MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: e923163
DOI: 10.12659/MSM.923163

Received: 2020.01.30
Accepted: 2020.03.22
Available online: 2020.04.20
Published: 2020.06.18

Puerarin Inhibits Endothelium-Dependent Contractions in Mouse Carotid Arteries

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 Mei Chen
BF 2 Li Xiang
FG 2 Guangliang Wu
EF 3 Yingdi Liao
AFG 1,2 Yefeng Cai

1 Department of Neurology, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou, Guangdong, P.R. China
2 School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, Hong Kong
3 Department of Neurology, The Second Clinical College of Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, P.R. China

Corresponding Author: Yefeng Cai e-mail: caiyefeng@126.com
Source of support: This project was supported by the Guangzhou Science and Technology Project (No. 201604020003), the Project of TCM Science and Technology in Guangdong Provincial Hospital of Chinese Medicine (No. YN2016ZD04)

Background: Many bioactive ingredients of medicinal plants are known to produce vaso-protective benefits. Puerarin is one of the major isoflavone glucosides found in the root of kudzu vine and it exerts an anti-inflammatory effect and many other pharmacological actions. However, the mechanism underlying the vascular effect of puerarin is incompletely understood. Therefore, the present study aims to examine how puerarin reduces endothelium-dependent contractions (EDCs) in mouse arteries.

Material/Methods: EDCs were evoked by acetylcholine (ACh) in isolated mouse carotid arteries with intact endothelium pretreated with Nω-NO2-L-Arg-OMe (L-NAME). The arteries were pretreated with puerarin and other pharmacological inhibitors before the addition of cumulative concentrations of ACh. The concentration of several prostaglandins (PGs) was measured by high performance liquid chromatography-coupled spectrometry (HPLC-MS).

Results: EDCs induced by ACh only presented in endothelium-intact arteries pretreated by L-NAME and EDCs were prevented by the treatment with cyclooxygenase (COX) inhibitor indomethacin (3 μmol/L) or thromboxane prostanoid receptor (TP receptor) antagonist S18886 (30 nmol/L). Acute 40-minute treatment with puerarin reduced EDCs in a concentration-dependent manner without affecting U46619-induced contraction. However, treatment with puerarin did not inhibit ACh-induced production of prostaglandins (PGs) in endothelium-intact arteries.

Conclusions: The present results show that puerarin is able to suppress EDCs in mouse carotid arteries, independent of inhibition of TP receptor or COX2-derived PGs.

MeSH Keywords: Cyclooxygenase 1 • Cyclooxygenase 2 • Drugs, Chinese Herbal • Endothelium, Vascular • Pueraria

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/923163

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Endothelium cells regulate the contractility of the underlying vascular smooth muscle through releasing several vasoactive substances, including both endothelium-derived relaxing factors (EDRFs) and endothelium-derived contracting factors (EDCFs). The balance between EDRFs and EDCFs maintains vascular homeostasis. Nitric oxide (NO), synthesized by endothelial nitric oxide synthase (eNOS) in endothelium is the best characterized EDRF. Upon diffusion from endothelial cells to vascular smooth muscle cells, NO activates guanylyl cyclase to raise cGMP levels and subsequently causes smooth muscle relaxation. Acetylcholine (ACh) has 2 effects on blood vessels. When ACh binds to M2 receptors on the vascular endothelium, it stimulates the formation of NO by NO synthase. The NO can then diffuse to smooth muscle cells, which caused vessel dilation. ACh can also bind to M3 receptors located in the endothelium. This activates the IP_3 pathway and stimulates calcium release by the sarcoplasmic reticulum, which leads to increased smooth muscle contraction. If the endothelium is intact, stimulation of the NO-cGMP pathway dominates over the activation of IP_3 pathway; therefore, ACh caused vasodilation [1,2]. Endothelium-dependent contractions (EDCs) exists in certain types of healthy blood vessels while hypertension, diabetes, and aging can reduce NO bioavailability and thus exaggerate EDCs, thereby contributing to vascular dysfunction under pathological conditions [3–7]. ACh can increase intracellular calcium concentration in endothelium to activate phospholipase A2 for the release of arachidonic acid; the latter is converted to form 5 prostanoids, some of which elicit EDCs. Arachidonic acid in mammalian cells is normally metabolized by cyclooxygenases (COXs), leading to production of thromboxane A_2 (TXA_2), prostaglandin D_2 (PGD_2), prostacyclin (PGI_2), and prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [5]. Both TXA_2 and $PGF_{2\alpha}$ are known to activate the TP receptor in vascular smooth muscle causing vascular contraction [8]. Although endothelial cells can produce other vasoconstrictors such as endothelin-1 and superoxide anions, prostanoids are generally regarded to be the major mediators of EDCs in blood vessels [9].

Two isoforms of COX exist in blood vessels [10]. But which isoform is dominant in mediating EDC remains controversial. COX-1 was reported to be the main source of EDCFs in mouse arteries and arteries of spontaneously hypertensive rats and diabetic rats [10,11]. However, under pathological conditions, the expression of COX-2 in blood vessels is upregulated and this isoform mediates a larger portion of EDCs [3,7,12].

Puerarin, also named 8-C-glucoside of daidzein (its chemical structure is shown in Figure 1A), is the major bioactive ingredient isolated from the root of the Pueraria lobata (Willd.) Ohwi, which is known as Gegen in Chinese traditional medicine [13]. Puerarin has been widely used to treat cardiovascular and cerebrovascular diseases in China [14,15]. Puerarin exerts several pharmacological actions including anti-inflammation, vasodilatation, neuroprotection, and antioxidant activity, suggesting that puerarin may possess a vaso-protective property [15]. But the effectiveness of puerarin on EDCs has not been reported and the pharmacological mechanisms are still unknown. Besides, a previous study reported the inhibition effect of puerarin on lipopolysaccharide (LPS)-induced COX-2 expression in RAW264.7 macrophage cells [16]. Another study reported a newly modified isoflavone based on puerarin inhibited LPS-induced production of arachidonic acid metabolites such as PGE_2 and leukotriene C4 (LTC4) [17].

In the present study, we used the NO synthase inhibitor L-NAME to inhibit NO production, thus unmasking EDCs in mouse carotid rings induced by acetylcholine. We showed that puerarin concentration-dependently inhibited EDCs.

Material and Methods

All experiments were performed on the common carotid arteries from adult (8-week-old) male C57BL/6 mice. The study conformed to the guidelines of the Animal Ethics Committee, Chinese University of Hong Kong, China.

Reagents and drugs

Ach (acetylcholine), indomethacin, L-NAME, phenylephrine (Phe) and U46619 (9,11-dideoxy11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$) were from Sigma-Aldrich Chemical. DuP-697 (5-bromo-2-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-thiophene) and VAS (valeryl salicylate) were from Cayman Chemical. S18886 (3-[(6-amino-(4-chlorobenzensulphonyl)-2-methyl5,6,7,8-tetrahydronapht]-1-yl) propionic acid) was from MedChemExpress. Celecoxib was purchased from Pfizer. ACh, S18886 and L-NAME were diluted in distilled water. And other drugs were diluted in dimethyl sulfoxide (DMSO; Sigma-Aldrich). The DMSO concentration was limited to less than 0.1%. Puerarin was purchased from Shanghai Yuan Ye Biotechnology Co., Ltd., China.

Carotid artery preparation

C57BL/6 mice were sacrificed by CO_2 inhalation. Common carotid arteries were dissected out and incubated in Kreb's solution (KHS; in mmol/L, NaCl 119, $NaHCO_3$ 25, $MgCl_2$ 1, KCl 4.7, D-glucose 11.1, $CaCl_2$ 2.5, and KH_2PO_4 1.2). After removing the adipose tissue and connective tissue, carotid artery was cut into rings about 1.0–1.3 mm in length. Some segments were fixed by 2 wires in chambers of a Multi Myograph System (610M, Danish Myo Technology A/S, Aarhus N, Denmark) filled with 5 mL KHS [5]. The chambers were aerated with fixed gas of 95% O_2 and 5% CO_2 and maintained at 37°C.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

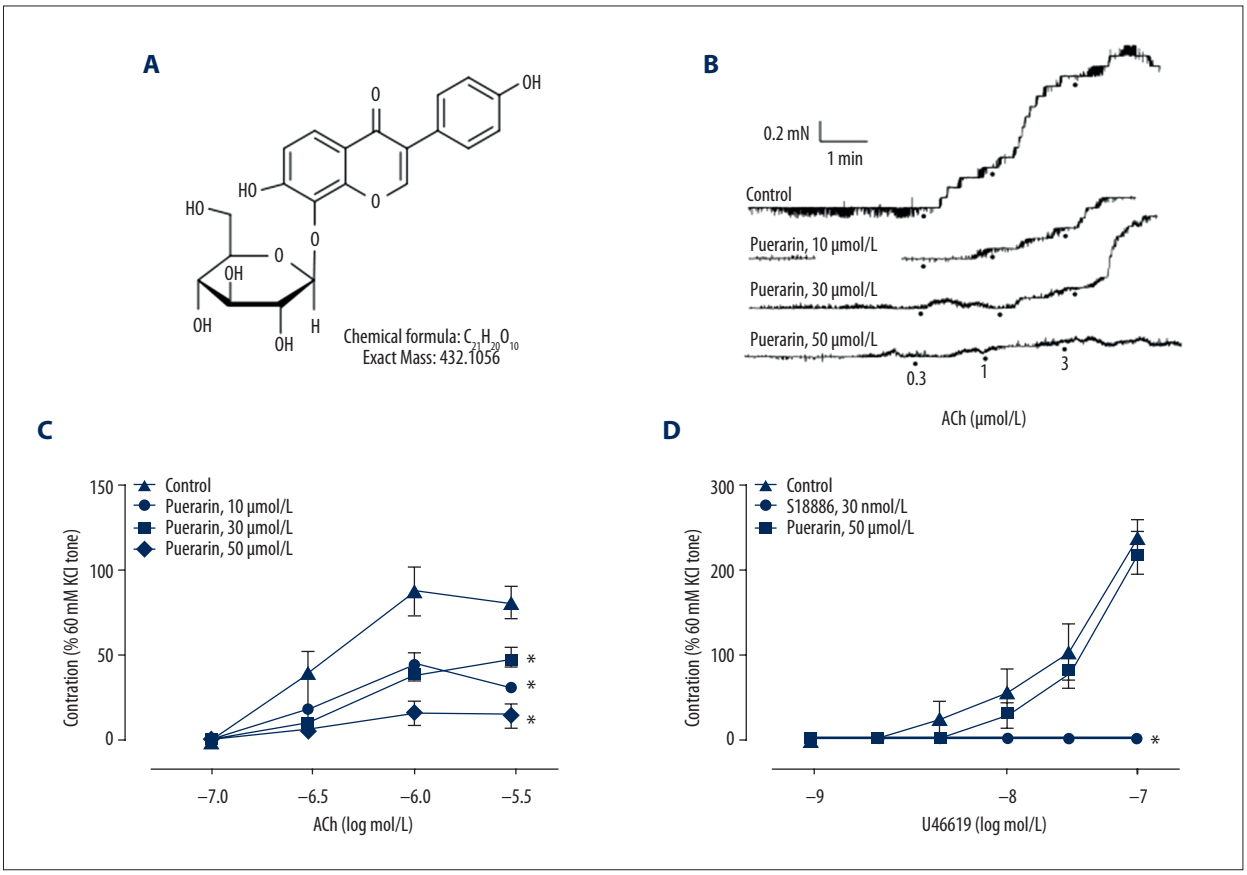

Figure 1. Puerarin inhibited endothelium-dependent contractions (EDCs) in mouse carotid artery. (**A**) The chemical structure of puerarin. (**B, C**) Concentration-dependent inhibitory effects of puerarin (10 μmol/L, n=5; 30 μmol/L, 50 μmol/L, n=6) on EDC compared to control (n=5). (**D**) The inhibitory effect of S18886 (30 nmol/L, n=4) but not puerarin (50 μmol/L, n=6) on U46619-induced contraction compared to control (n=5). The results are means±standard error of the mean (SEM). * *P*<0.05 *vs.* control.

Isometric force measurement

The carotid artery rings were mounted in myograph and adjusted to the resting tension of ~1 mN and maintained for 30 minutes. Thereafter, each ring was contracted first with 60 mM KCl to confirm artery's integrity. Then we washed the chambers by KHS 4 times, and allowed the rings to equilibrate for 10 minutes. In some rings, the endothelium was removed gently with mechanical rolling by a tungsten wire [5,8]. To test the effectiveness, we tested if ACh could inhibit Phe induced contraction in some rings. For detection of EDC, all rings were exposed to L-NAME (100 μmol/L) for 30 minutes to abolish the production of endothelium-derived NO before cumulative additions of ACh (0.3 μmol/L to 3 μmol/L). Puerarin, COX inhibitors, and S18886 were added to bathing solution 10 minutes before application of L-NAME or U46619 (1 nmol/L to 100 nmol/L).

High-performance liquid chromatography – coupled mass spectrometry measurement of prostaglandins (PGs)

After being pretreated with 100 μmol/L L-NAME with or without puerarin at 37°C, the entire mouse carotid arteries were moved to microcentrifuge tubes that contained 100 μL KHS and ACh (10 μmol/L) at 37°C. Three minutes after, arteries and incubation solutions were frozen and stored at –80°C for the determination of released prostaglandins (PGs) by high performance liquid chromatography-coupled spectrometry (HPLC-MS).

After vacuum dried with 10 μL of internal standard, samples were derivatized as described previously [18]. Measurement was carried out on liquid chromatography (LC, Ultimate 3000, Dionex, Sunnyvale, CA, USA) coupled with a Thermo ScientificTM TSQ QuantivaTM triple quadrupole mass spectrometry (MS, Thermo Scientific, San Jose, CA, USA). Separation was performed on a Waters BEH C18 column (2.1 mm i.d. ×100 mm, 1.7 μm particle size, Waters). For the LC part, column temperature was maintained at 30°C. Flow rate was 0.2 mL/minute. Injection

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

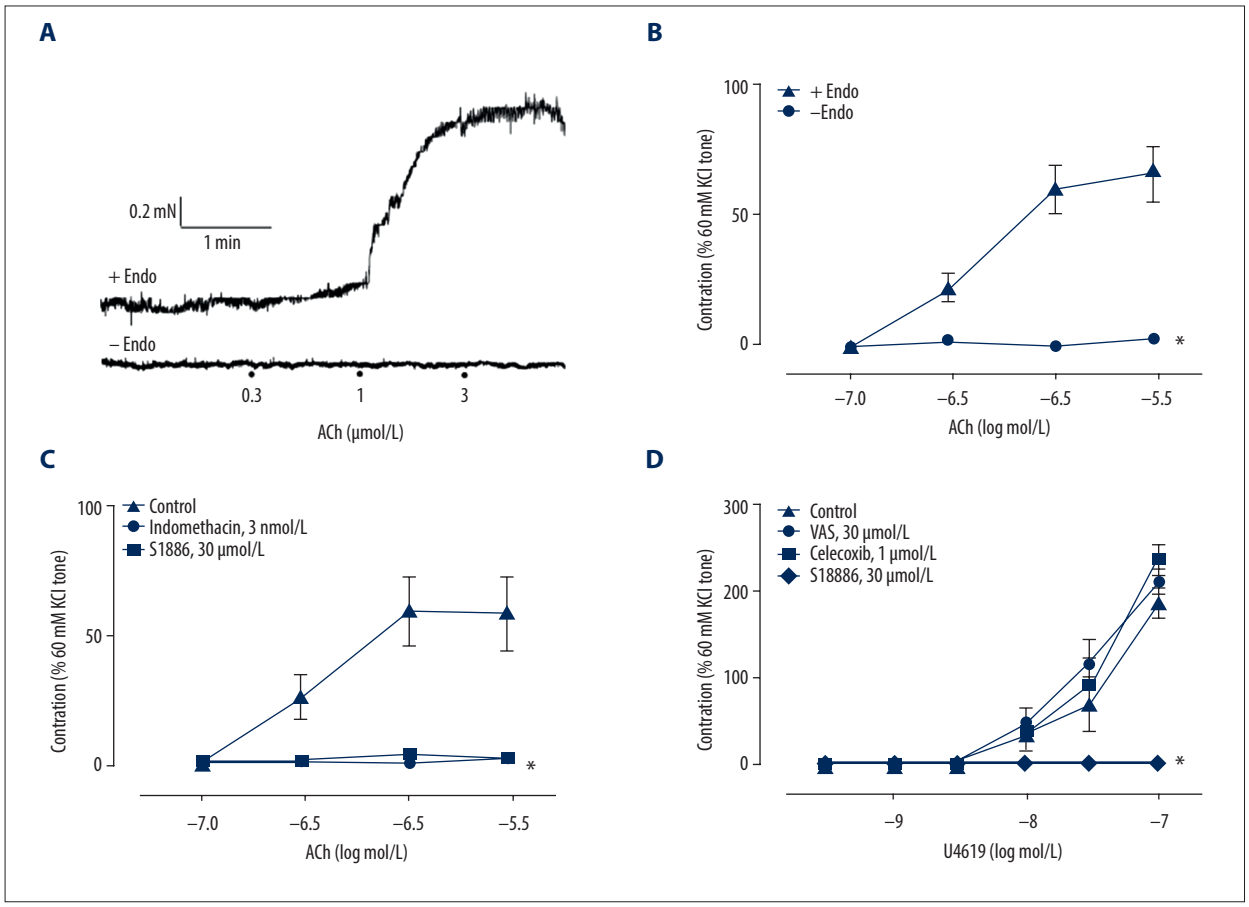

Figure 2. Cyclooxygenase (COX) and thromboxane prostanoid (TP) receptor mediated endothelium-dependent contractions (EDCs). (**A**) Original recordings showing acetylcholine (ACh)-induced contractions only in mouse carotid arteries with intact endothelium. (**B**) ACh-induced concentration-dependent contractions in L-NAME-treated arteries with endothelium (+Endo [n=8] *vs.* –Endo [n=6]). (**C**) The inhibitory effects of non-selective COX inhibitor, indomethacin (3 μmol/L, n=6) and the TP receptor antagonist, S18886 (30 nmol/L, n=6) on ACh-induced contraction compared to control (n=5). (**D**) The effects of COX-1 inhibitor valeryl salicylate (VAS) (30 μmol/L, n=6), COX-2 inhibitor celecoxib (1 μmol/L, n=6), and TP receptor antagonist S18886 (30 nmol/L, n=4) on TP receptor agonist U46619-induced contraction compared to control (n=4). The results are means±standard error of the mean (SEM). * $P<0.05$ *vs.* control.

volume was 10 μL. Mobile phase A was water with 10 mmol/L ammonium acetate and 0.1% formic acid, phase B was 95% acetonitrile (acetonitrile/H_2O=95: 5 (v/v)) with 10 mmol/L ammonium acetate and 0.1% formic acid. For the MS part, Sheath Gas Flow Rate, 40 arb; Aux Gas Flow Rate, 10 arb; Spray Voltage, 3.5 kV in positive ion mode; Ion Transfer Tube Temp., 350°C and Vaporizer Temp., 320°C.

Data analysis

Results are presented as means±standard error of the mean (SEM). Concentration-response curves were fixed to a sigmoidal curve using non-linear regression with the assistance of the statistical software GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). And 2-way ANOVA were used to analysis the data. $P<0.05$ was considered significantly different.

Results

Puerarin inhibited EDC in C57BL/6 mouse carotid artery

Pretreated with L-NAME, EDCs induced by ACh were only present in mouse common carotid arteries with intact endothelium (Figure 2A, 2B). The traces in Figure 1B show that acute 40-minute treatment with puerarin concentration-dependently inhibited ACh-induced EDCs (10–50 μmol/L, Figure 1C). The concentration of puerarin was decided based on our preliminary experiments and previous study [19]. By contrast, puerarin (50 μmol/L) did not inhibit contractions evoked by the thromboxane prostanoid (TP) receptor agonist U46619, while the TP receptor antagonist S18886 (30 nmol/L) abolished U46619-induced contraction in mouse arteries (Figure 1D).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

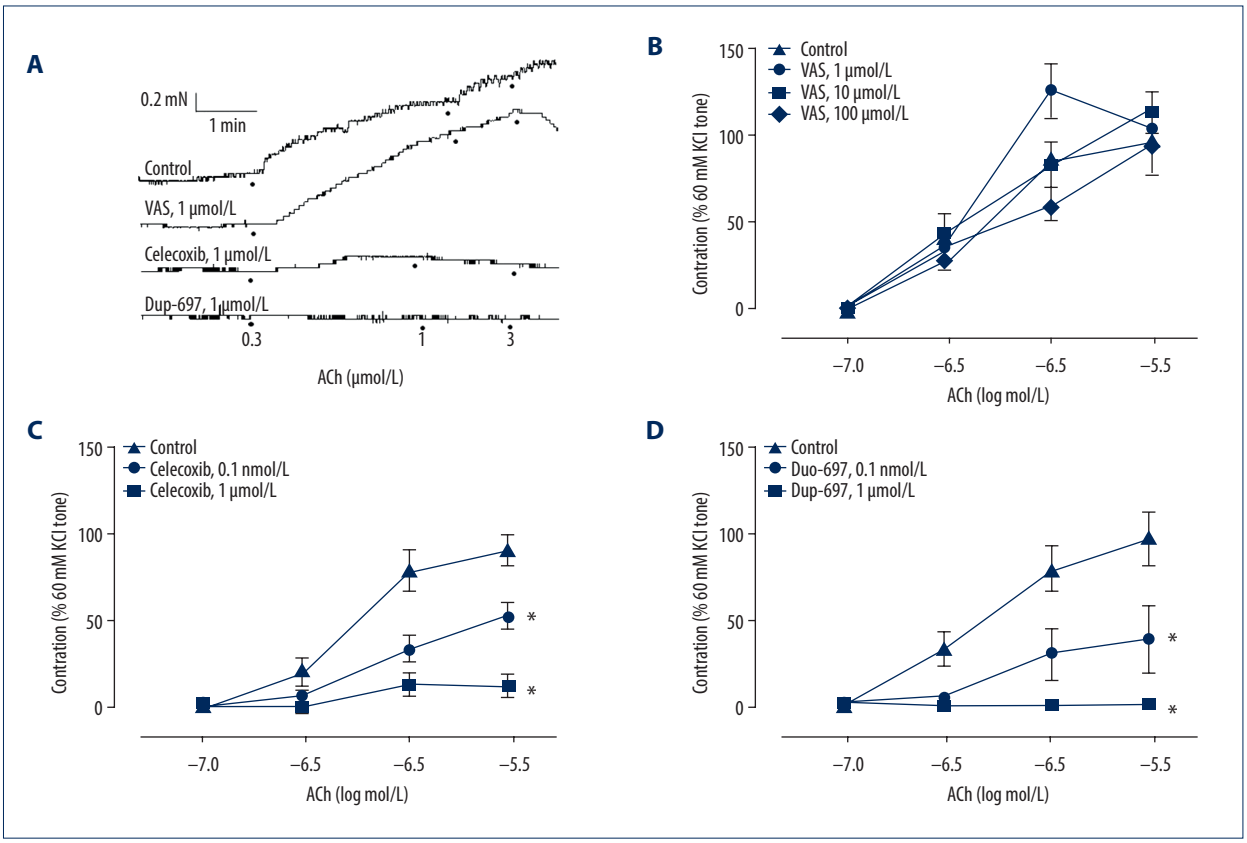

Figure 3. Cyclooxygenase-2 (COX-2) not COX-1 mediated endothelium-dependent contractions (EDCs) in mouse carotid artery. (**A**) Original recordings showing the effect of COX-1 and COX-2 inhibitors on EDCs. (**B**) Lack of effect of the selective COX-1 inhibitor VAS (1 to 100 μmol/L, n=4) on acetylcholine (ACh)-induced contractions compared with control (n=7). (**C**) Concentration-dependent inhibitory effect of celecoxib (0.1 to 1 μmol/L, n=7) on EDCs compared with control (n=6). (**D**) Concentration-dependent inhibitory effect of Dup-697 (0.1 to 1 μmol/L, n=6) on ACh-induced contractions compared with control (n=6). The results are means±standard error of the mean (SEM). * *P*<0.05 versus control.

Cyclooxygenase (COX) and TP receptor mediated EDC in C57BL/6 mice

Pretreated with L-NAME, ACh elicited EDCs in mouse carotid arteries with a maximum response of approximately 70% of the contractions induced by 60 mmol/L KCl (Figure 2B). ACh failed to induce contractions in arteries without endothelium (Figure 2A, 2B). ACh also failed to contract endothelium-intact arteries that were pretreated with either indomethacin (3 μmol/L) or S18886 (30 nmol/L) (Figure 2C).

COX-2 not COX-1 mediated EDC in C57BL/6 mice

In order to determine which COX isoform mediates EDCs, both COX-1 selective inhibitor (VAS) and COX-2 selective inhibitor (celecoxib) were used. Neither of them affected U46619-induced contractions which was reversed by S18886 at 30 nmol/L (Figure 2D). Treatment with VAS (1 to 100 μmol/L) did not affect the ACh-induced contractions (Figure 3A, 3B). On the contrary, EDCs were largely attenuated or abolished in carotid arteries following treatment with 2 different COX-2 inhibitors, celecoxib (0.1 to 1 μmol/L) and DuP-697 (0.1 to 1 μmol/L) (Figures 3A, 3C, 3D).

Effects of puerarin on acetylcholine-induced release of prostaglandins

In endothelium-intact mouse common carotid arteries treated with L-NAME (100 μmol/L), ACh at 10 μmol/L significantly elevated the level of 4 prostaglandins, i.e., $PGF_{2\alpha}$, TXA_2 (detected as TXB_2), PGE_2, and PGD_2 in bathing solution as measured by HPLC-MS (Figure 4). The ACh-induced release of the 4 prostaglandins was not affected by pre-treatment with puerarin 50 μmol/L (Figure 4).

Discussion

In this study, we mainly found that 1) puerarin inhibited ACh-induced EDCs in mouse carotid arteries, 2) puerarin did not

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

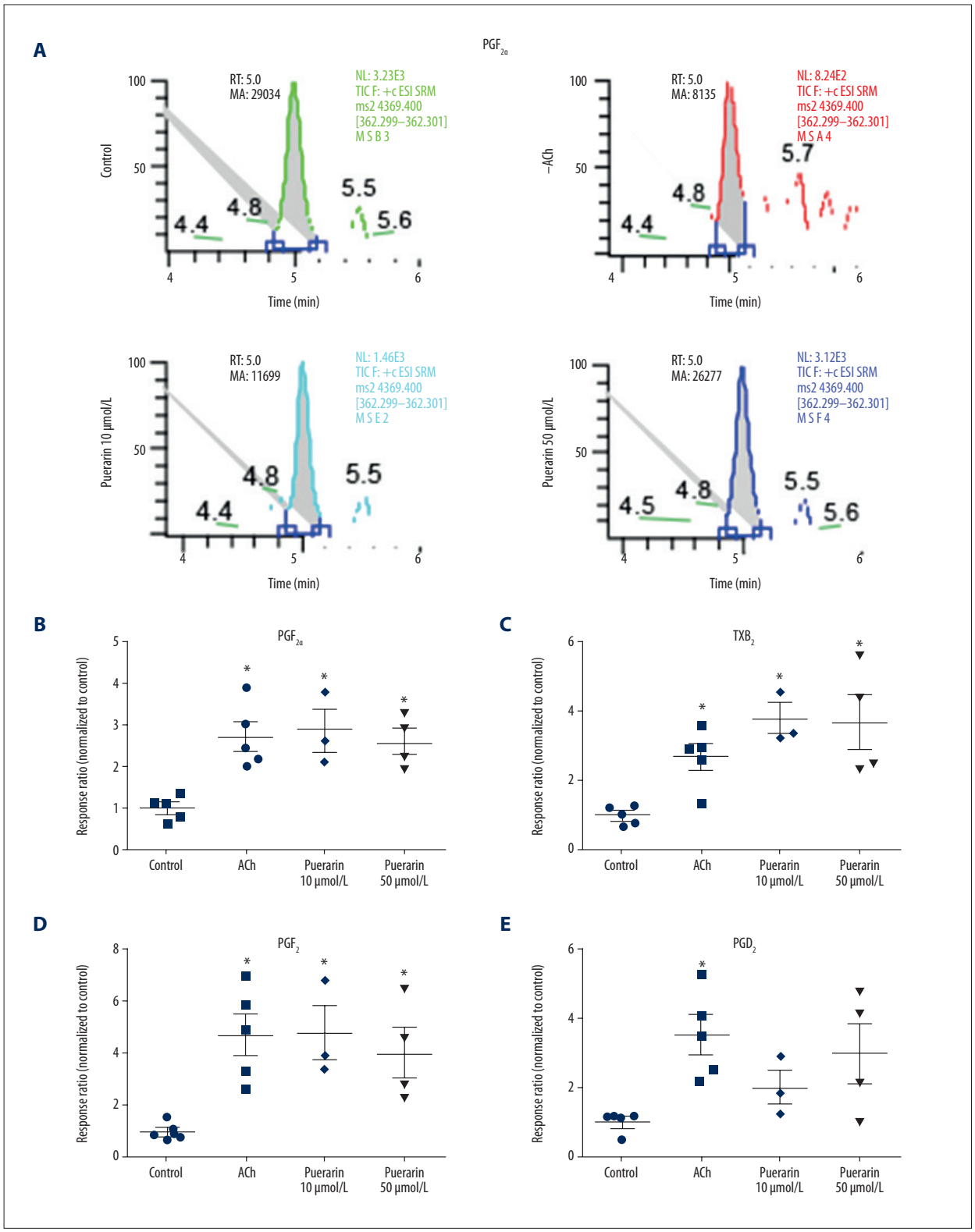

Figure 4. (A–E) Effects of puerarin on acetylcholine (ACh)-induced release of prostaglandins. High performance liquid chromatography-coupled spectrometry (HPLC-MS) measurement of $PGF_{2\alpha}$, TXA_2, PGE_2, and PGD_2 in the bath solution of L-NAME (100 μmol/L)-treated mouse carotid arteries with and without exposure to ACh (10 μmol/L, n=5), or to ACh plus puerarin (10 μmol/L, n=3; 50 μmol/L, n=4). The results are means ± standard error of the mean (SEM). * P<0.05 *vs.* control.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

affect TP-receptor agonist-induced contractions, 3) COX-2 selective inhibitors but not COX-1 selective inhibitor inhibited or abolished EDCs, and 4) puerarin did not affect ACh-induced production of PGE_2, PGD_2, $PGF_{2\alpha}$, and TXA_2 in mouse carotid rings.

Previous *ex vitro* and *in vivo* studies showed that puerarin improved vascular function in rodent models of hypertension [20]. Puerarin was reported to augment endothelium-independent relaxations in the porcine coronary artery through a cyclic AMP-dependent mechanism [21]. Puerarin protected against vascular dysfunction induced by angiotensin II, probably through suppressing generation of reactive oxygen species [22,23]. Puerarin inhibited LPS-induced injury in human endothelial cells through inhibiting NF-κB activity [24] and puerarin could lower blood pressure in spontaneously hypertensive rats probably by increasing the level and function of NO [25]. However, these aforementioned studies mainly focused on the beneficial effect of puerarin on endothelium-dependent relaxations in relation to the balance between NO and reactive oxygen species. It is well established that endothelial dysfunction is closely associated with the disrupted balance between endothelium-derived relaxing and contracting factors [4]. The loss of endothelium-derived NO in hypertension unmasked the vaso-damaging effect of reactive oxygen species in the arteries as reflected by exaggerated EDCs [4,6]. Although puerarin was shown to exert several beneficial effects including enhanced endothelium-dependent relaxations in blood vessels, its effect on EDCs was unknown. The present study was probably the first one to show the inhibitory effect of puerarin on ACh-induced EDCs as additional mechanism by which this compound protects vascular function.

Both COX-1 and COX-2 were responsible for chemical conversion of arachidonic acid into the end products, the 5 prostanoids [10]. Some studies suggest that COX-1 is the main player in the generation of EDCFs under both physiological and pathological conditions [26–28]. Whereas, other studies showed that the expression of COX-2 in blood vessels in hypertension and ageing was elevated and COX-2 is the major source of EDCFs [3,5,12]. It appeared that the species and anatomic location of the arteries dissected for *ex vivo* study might affect the relative role of COX-1 and COX-2 in the generation of EDCFs. The present study used C57BL/6 mouse carotid arteries for functional examination to determine the role of COX isoforms in ACh-induced EDCs. Our results showed that COX-2 selective inhibitors celecoxib and DUP-697, but not COX-1 selective inhibitor VAS in attenuated or reversed ACh-induced EDCs, indicating that COX-2 was the most likely isoform to mediate generation of EDCFs and associated EDCs in mouse carotid arteries.

Arachidonic acid was metabolized by COX to generate different kinds of prostanoids, such as, $PGF_{2\alpha}$, PGD_2, PGE_2, TXA_2, and PGI_2 [5]. Previous study indicated that PGI_2 did not induce any contraction even at a high concentration in both mice carotid and hamster aorta [5,8]. To determine whether puerarin-induced inhibition of EDCs was due its effect on COX-2-derived prostanoids, we measured the other 4 prostanoids $PGF_{2\alpha}$, PGE_2, PGD_2, and TXA_2. These molecules were known to involve the regulation of vascular homeostasis by modulating vascular tone of blood vessels [8,29]. We found that ACh was able to trigger increase of the 4 prostanoids in L-NAME-treated endothelium-intact mouse carotid arteries as determined by the HPLC-MS method. However, pretreatment with puerarin at a concentration that reduced EDCs failed to inhibit the ACh-induced production of the prostanoids, suggesting that the inhibitory effect of puerarin on EDCs might be unrelated to COX-2-derived vasoconstrictive prostanoids. Like U46619-induced contraction, ACh-induced EDC could be abolished by the pretreatment with TP receptor antagonist, S18886. However, puerarin did not affect TP receptor-mediated contraction, suggesting that puerarin may not directly interfere with TP receptors in mouse carotid arteries. It was reported that puerarin could activate endothelial eNOS [30]. Another study indicated that puerarin protected against endothelial dysfunction by increasing phosphor-eNOS at Ser1177 [20], which might explain the abolition of EDCs. Previously, it was also reported that puerarin could inhibit calcium influx [31], which might also partly contribute to the inhibition effect of EDCs. Further work needs to be done to explore the mechanism.

Conclusions

In summary, puerarin was effective to inhibit EDCs in a concentration-dependent fashion without affecting TP receptor-mediated contraction in mouse carotid rings. The HPLC-MS showed puerarin was ineffective in inhibiting ACh-induced production of prostanoids, indicating that puerarin might exert a non-selective effect to suppress ACh-induced COX-2-related EDCs in mouse common carotid arteries.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Grueb M, Reinthal E, Rohrbach JM et al: Muscarinic acetylcholine receptor subtypes in human corneal epithelium and endothelium. Graefes Arch Clin Exp Ophthalmol, 2006; 244(9): 1191–95
2. Vanhoutte PM, Shimokawa H, Feletou M et al: Endothelial dysfunction and vascular disease – a 30th anniversary update. Acta Physiol (Oxf), 2017; 219(1): 22–96
3. Wong WT, Tian XY, Chen YC et al: Bone morphogenic protein-4 impairs endothelial function through oxidative stress-dependent cyclooxygenase-2 upregulation: implications on hypertension. Circ Res, 2010; 107(8): 984–91
4. Wong WT, Wong SL, Tian XY et al: Endothelial dysfunction: The common consequence in diabetes and hypertension. J Cardiovasc Pharmacol, 2010; 55(4): 300–7
5. Wong SL, Leung FP, Lau CW et al: Cyclooxygenase-2-derived prostaglandin F2alpha mediates endothelium-dependent contractions in the aortae of hamsters with increased impact during aging. Circ Res, 2009; 104(2): 228–35
6. Dong J, Wong SL, Lau CW et al: Calcitriol protects renovascular function in hypertension by down-regulating angiotensin II type 1 receptors and reducing oxidative stress. Eur Heart J, 2012; 33(23): 2980–90
7. Tian XY, Wong WT, Leung FP et al: Oxidative stress-dependent cyclooxygenase-2-derived prostaglandin $f_{(2\alpha)}$ impairs endothelial function in renovascular hypertensive rats. Antioxid Redox Signal, 2012; 16(4): 363–73
8. Liang C, Zhang Y, Zhuo D et al: Endothelial cell transient receptor potential channel C5 (TRPC5) is essential for endothelium-dependent contraction in mouse carotid arteries. Biochem Pharmacol, 2019; 159: 11–24
9. Baretella O, Vanhoutte PM: Endothelium-dependent contractions: Prostacyclin and endothelin-1, partners in crime? Adv Pharmacol, 2016; 77: 177–208
10. Félétou M, Huang Y, Vanhoutte PM: Vasoconstrictor prostanoids. Pflugers Arch, 2010; 459(6): 941–50
11. Liu D, Liu B, Luo W et al: A vasoconstrictor response to COX-1-mediated prostacyclin synthesis in young rat renal arteries that increases in prehypertensive conditions. Am J Physiol Heart Circ Physiol, 2015; 309(5): H804–11
12. Virdis A, Bacca A, Colucci R et al: Endothelial dysfunction in small arteries of essential hypertensive patients: Role of cyclooxygenase-2 in oxidative stress generation. Hypertension, 2013; 62(2): 337–44
13. Li XF, Yuan T, Xu H et al: Whole-cell catalytic synthesis of puerarin monoesters and analysis of their antioxidant activities. J Agric Food Chem, 2019; 67(1): 299–307
14. Zheng QH, Li XL, Mei ZG et al: Efficacy and safety of puerarin injection in curing acute ischemic stroke: A meta-analysis of randomized controlled trials. Medicine (Baltimore), 2017; 96(1): e5803
15. Zhou YX, Zhang H, Peng C: Puerarin: A review of pharmacological effects. Phytother Res, 2014; 28(7): 961–75
16. Hu W, Yang X, Zhe C et al: Puerarin inhibits iNOS, COX-2 and CRP expression via suppression of NF-κB activation in LPS-induced RAW264.7 macrophage cells. Pharmacol Rep, 2011; 63(3): 781–89
17. Xiang Y, Wei X, Chen L et al: Anti-inflammatory effect of acetylpuerarin on eicosanoid signaling pathway in primary rat astrocytes. J Mol Neurosci, 2014; 52(4): 577–85
18. Bian X, Sun B, Zheng P et al: Derivatization enhanced separation and sensitivity of long chain-free fatty acids: Application to asthma using targeted and non-targeted liquid chromatography-mass spectrometry approach. Anal Chim Acta, 2017; 989: 59–70
19. Deng Y, Ng ES, Yeung JH et al: Mechanisms of the cerebral vasodilator actions of isoflavonoids of Gegen on rat isolated basilar artery. J Ethnopharmacol, 2012; 139(1): 294–304
20. Li X, Lin Y, Zhou H et al: Puerarin protects against endothelial dysfunction and end-organ damage in Ang II-induced hypertension. Clin Exp Hypertens, 2017; 39(1): 58–64
21. Yeung DK, Leung SW, Xu YC et al: Puerarin, an isoflavonoid derived from *Radix puerariae*, potentiates endothelium-independent relaxation via the cyclic AMP pathway in porcine coronary artery. Eur J Pharmacol, 2006; 552(1–3): 105–11
22. Lu XL, Liu JX, Wu Q et al: Protective effects of puerarin against Aβ40-induced vascular dysfunction in zebrafish and human endothelial cells. Eur J Pharmacol, 2014; 732: 76–85
23. Chen G, Pan SQ, Shen C et al: Puerarin inhibits angiotensin II-induced cardiac hypertrophy via the redox-sensitive ERK1/2, p38 and NF-κB pathways. Acta Pharmacol Sin, 2014; 35(4): 463–75
24. Deng HF, Wang S, Li L et al: Puerarin prevents vascular endothelial injury through suppression of NF-κB activation in LPS-challenged human umbilical vein endothelial cells. Biomed Pharmacother, 2018; 104: 261–67
25. Shi W, Yuan R, Chen X et al: Puerarin reduces blood pressure in spontaneously hypertensive rats by targeting eNOS. Am J Chin Med, 2019; 47(1): 19–38
26. Liu B, Luo W, Zhang Y et al: Involvement of cyclo-oxygenase-1-mediated prostacyclin synthesis in the vasoconstrictor activity evoked by ACh in mouse arteries. Exp Physiol, 2012; 97(2): 277–89
27. Li S, Liu B, Luo W et al: Role of cyclooxygenase-1 and -2 in endothelium-dependent contraction of atherosclerotic mouse abdominal aortas. Clin Exp Pharmacol Physiol, 2016; 43(1): 67–74
28. Zhu N, Liu B, Luo W et al: Vasoconstrictor role of cyclooxygenase-1-mediated prostacyclin synthesis in non-insulin-dependent diabetic mice induced by high-fat diet and streptozotocin. Am J Physiol Heart Circ Physiol, 2014; 307(3): H319–27
29. Félétou M, Huang Y, Vanhoutte PM: Endothelium-mediated control of vascular tone: COX-1 and COX-2 products. Br J Pharmacol, 2011; 164(3): 894–912
30. Hwang YP, Kim HG, Hien TT et al: Puerarin activates endothelial nitric oxide synthase through estrogen receptor-dependent PI3-kinase and calcium-dependent AMP-activated protein kinase. Toxicol Appl Pharmacol, 2011; 257(1): 48–58
31. Wang K, Zhu X, Zhang K et al: Neuroprotective effect of puerarin on glutamate-induced cytotoxicity in differentiated Y-79 cells via inhibition of ROS generation and Ca(2+) influx. Int J Mol Sci, 2016; 17(7): pii: E1109

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]