The Protective Effect of Puerarin on Myocardial Infarction Reperfusion Injury (MIRI): A Meta-Analysis of Randomized Studies in Rat Models

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

ABCDEFG **Huang Wenjun***
ABCD **Wen Jing***
ABCG **Li Tao**
DEF **Mao Liang**
DEFG **Yang Yan**
ABC **Zeng Xiaorong**
ABCDEFG **Zhou Rui**

Key Laboratory of Medical Electrophysiology, Ministry of Education, Collaborative Innovation Center for Prevention and Treatment of Cardiovascular Disease/Institute of Cardiovascular Research, Sichuan Medical University, Luzhou, Sichuan, P.R. China

* Co-first author

Corresponding Author: Zhou Rui, e-mail: zhouhuaxizhu@126.com

Background: Although puerarin is generally considered as a protective agent for cardio-cerebrovascular diseases, the exact effect on reducing myocardial infarction reperfusion injury (MIRI) is not well understood. This study aimed to pool previous randomized controlled studies based on rat models to evaluate the effects of puerarin on MIRI.

Material/Methods: Relevant studies were searched among PubMed, Embase, Medline, and CNKI (China National Knowledge Infrastructure). To assess the therapeutic effects of protective effects of puerarin on myocardial infarction reperfusion injury, the outcome indicators which were reported in at least 3 original studies were extracted and pooled, including size of myocardial ischemia (MIS) and myocardial infarction (MIN), creatine kinase (CK), methylene dioxyamphetamine (MDA), and superoxide dismutase (SOD).

Results: Administration of puerarin could effectively reduce the size of MIN after MIR (mean difference: −29.20, 95%CI: −44.90 to −13.51, p=0.0003). Puerarin directly led to decreased CK (mean difference: −6.89, 95%CI: −9.40 to −4.38, p=0.00001) and MDA (mean difference: −2.41, 95%CI: −3.14 to −1.68, p<0.00001) and increased serum SOD (mean difference: 63.97, 95%CI: 38.19 to 89.75, p<0.00001).

Conclusions: Puerarin might have a protective effect in myocardial tissues during MIRI through increasing SOD and decreasing CK and MDA. However, more animal studies and randomized controlled clinical trials are required to confirm these results.

MeSH Keywords: Meta-Analysis as Topic • Myocardial Infarction • Myocardial Reperfusion Injury

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/894312

📄 1811   🎞 1   📊 6   📖 25

Background

Acute myocardial ischemia and myocardial infarction are the leading cause of morbidity and mortality of the world [1]. Early reperfusion to the ischemic area is necessary to rescue the ischemic myocardium. However, reperfusion is a 'double edged sword', since it may also accelerate and generate additional damage by introduction of oxidative stress and inflammation rather than restoration of normal function [2]. Oxygen radicals, calcium overloading, and neutrophils are the major mediators of reperfusion injury [1,3]. The oxygen radicals are released from injured endothelial cells and myocytes in the ischemic area and also from neutrophils moved to the area. The oxygen radicals become activated due to reperfusion and cause membrane damage, thereby leading to calcium overloading. The neutrophils entered the ischemic area also release inflammatory mediators, leading to microvascular obstruction and the no-reflow phenomenon [1]. The reversible dysfunction and irreversible necrosis associated with reperfusion are collectively called reperfusion injury and is one of the major problems for treatment of myocardial infarction [4–6].

Puerarin (7,4-dihydroxyisoflavone-8β-glucopyranoside) is a major natural compound extracted from the kudzu root (Pueraria lobota (Wild.) Howe), a famous traditional Chinese medicine [7]. This drug is widely prescribed for patients with cardio-cerebrovascular diseases in China. Previous studies also showed that puerarin has some therapeutic effects on diabetes mellitus [8], cerebral ischemia [9], myocardial ischemia [10], hypertension [11] and arteriosclerosis [12]. Studies showed the therapeutic effect of puerarin is closely related to its antioxidant role. Therefore, it acts as a scavenger of active oxygen radicals [13]. In addition, puerarin can also improve endothelial function through stimulating production of nitric oxide, phosphorylation of endothelial nitric oxide synthase and inhibiting cellular factors, such as C-reactive protein and adhesive molecules [7].

Although puerarin is generally considered as a protective agent for cardio-cerebrovascular diseases, the exact effect on reducing myocardial infarction reperfusion injury (MIRI) is not well understood. Previous animal based studies are usually small and thus had low statistical power. Therefore, this study aims to pool previous randomized controlled studies based on rat model to evaluate the effects of puerarin in MIRI.

Material and Methods

Literature search

Relevant studies were searched among PubMed, Embase, Medline and CNKI (China National Knowledge Infrastructure).

The following the terms and strategy were applied to search relevant studies: ("puerarin") AND ("myocardial infarction reperfusion injury") AND ("rat"). To avoid missing qualified studies, references lists of included studies, relevant reviews and meta-analysis were manually searched. No language restrictions were set during searching.

Inclusion and exclusion criteria

Studies included for this meta-analysis have to meet the following criteria simultaneously: (1) randomized studies assessed the protective effects of puerarin supplementation on myocardial infarction reperfusion injury in rat model; (2) puerarin is either administrated before myocardial ischemia reperfusion or before myocardial ischemia; (3) the exact outcome data could be extract from original studies. Studies meeting any the following criteria were excluded: (1) studies based on other animal models; (2) case report, animal studies or review; (3) duplicate studies or detailed data could not be extracted.

Data extraction

The following basic information was extracted from original studies: surname of the first author; year of publication; animal model; methods of myocardial ischemia; number of rats in and experimental design of experimental and control group; timing of puerarin administration and the outcome indicators measured. To assess the therapeutic effects of protective effects of puerarin on myocardial infarction reperfusion injury, the outcome indicators which were reported in at least three original studies were extracted and pooled, including size of myocardial ischemia (MIS) and myocardial infarction (MIN), creatine kinase (CK), methylene dioxyamphetamine (MDA) and superoxide Dismutase (SOD). TTC staining was performed in the original studies to confirm the ischemia area. Size of MIN is defined as the weight of infarcted myocardium/weight ischemic myocardium ×100% or the area of infarcted area/whole myocardium ×100%. Size of MIS is defined as the weight ischemic myocardium/weight of whole heart. If the studies reported outcome with different units, unit conversion (based on unit mentioned above) were performed before pooling the data. Two scholars independently performed data extraction. If the studies designed different dose groups of puerarin, each dose group was considered as an individual experimental arm. A third author was responsible for cross check of the data. Any disagreements were solved by discussion and consensus.

Data analysis

Data integration and analysis is based on Review Manager 5.3 (the Cochrane Collaboration). All outcome analyzed are continuous variables. Thus, the weighted mean different (WMD) and
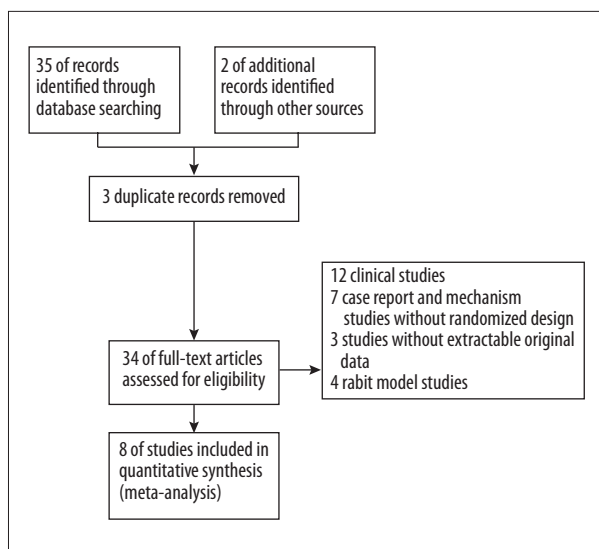
Figure 1. The searching and screening process.

the 95% confidence intervals (CI) were calculated. Chi-square based Q test and I^2 was used to assess between study heterogeneity. $p < 0.1$ in Q test or $I^2 > 50\%$ indicates significantly heterogeneity. A random effect model (DerSimonian and Laird method) was used if significant between study heterogeneity detected. Otherwise, the fixed effect model based on Mantel-Haenszel method was applied. For the pooled results, $p < 0.5$ in Z test was considered statistically significant.

Results

Characteristics of Included Studies

Through searching in relevant databases, a total of eight studies were included in this meta-analysis [14–21]. The general searching and screening process is summarized in Figure 1 and their basic characteristics are given in Table 1. The eight studies were published from 2006 to 2013. All of model of MIS was induced by ligation of left anterior descending coronary

Table 1. The key characteristics of studies included.

Study	Animal model	Method of MIS	No. animals		Experimental design		Timing of PUE administration	Outcome measured
			E	C	E	C		
Gao 2006	Rat	Ligation of LAD	11	11	PUE (100 mg/kg) + IR	I/R	Before MIS	Size of MIS, size of MIN, CK, TnT, cell apoptosis
Bao 2007	Rat	Ligation of LAD	20	10	PUE (100/200 mg/kg) +IR	I/R	Before MIS	CK, MDA, SOD, NO
Gao 2007	Rat	Ligation of LAD	11	11	PUE (100 mg/kg) +IR	I/R	Before MIS	Size of MIS, size of MIN, CK, TnT, cell apoptosis
Wang 2008	Rat	Ligation of LAD	20	20	IR+PUE (100 mg/kg)	I/R	Before MIR	Size of MIS, LDH, CK, SOD, MDA
Lu 2009a	Rat	Ligation of LAD	6	6	PUE (100 mg/kg) +IR	I/R	Before MIS	Size of MIS, size of MIN, CK, ET, NO
Lu 2009b	Rat	Ligation of LAD	6	6	IR+PUE (100 mg/kg)	I/R	Before MIR	Size of MIS, size of MIN, CK, ET, NO
Jia 2010	Rat	Ligation of LAD	24	8	IR+PUE (2/5/10 mg/kg)	I/R	Before MIR	SOD, MDA, GSH, GSH-Px
Pan 2010	Rat	Ligation of LAD	12	12	IR+PUE (20 mg/kg)	I/R	Before MIR	CK, MPO, MDA
Li 2013	Rat	Ligation of LAD	30	10	IR+PUE (2/5/10 mg/kg)	I/R	Before MIR	Size of MIN, CK, LDH, NO, XO, SOD, MDA, GSH and GSH-Px

MIR – myocardial ischemia reperfusion; MIS – myocardial ischemia; MIN – myocardial infarction; LAD – left anterior descending coronary artery; PUE – Puerarin; I/R – ischemia-reperfusion; CK – creatine kinase; MDA – methylene dioxyamphetamine; SOD – superoxide dismutase; GSH – glutathione; GSH-Px – glutathione peroxidase; MPO – myeloperoxidase; NO – nitrogen monoxide; TnT – cardiac troponin T; ET – endothelin; XO – xanthineocidase; N.A. – not available; E – experimental; C – control.
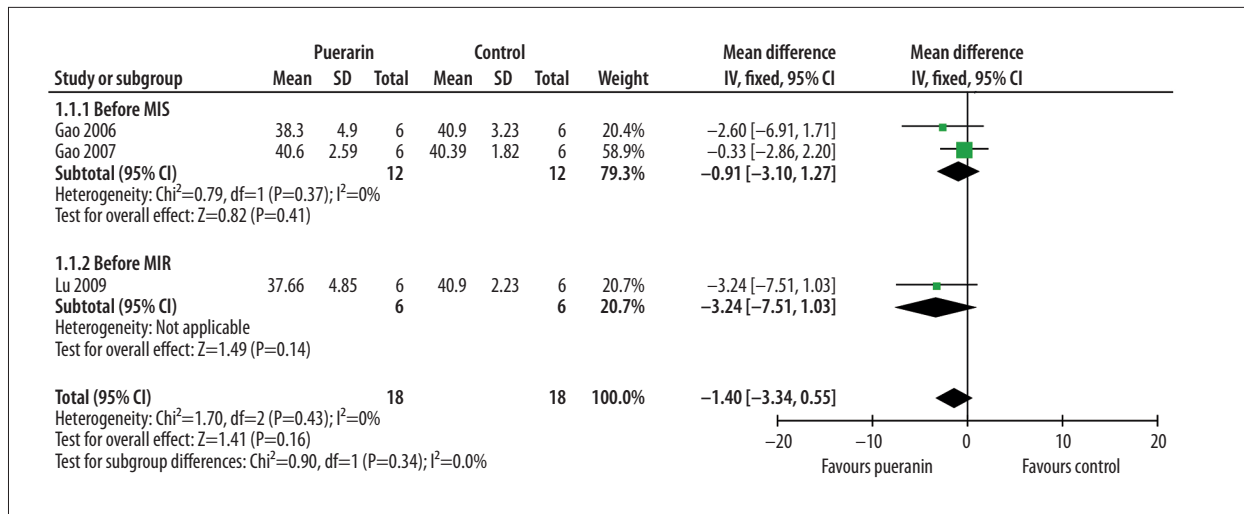
Figure 2. The effect of puerarin on size of myocardial ischemia (MIS).
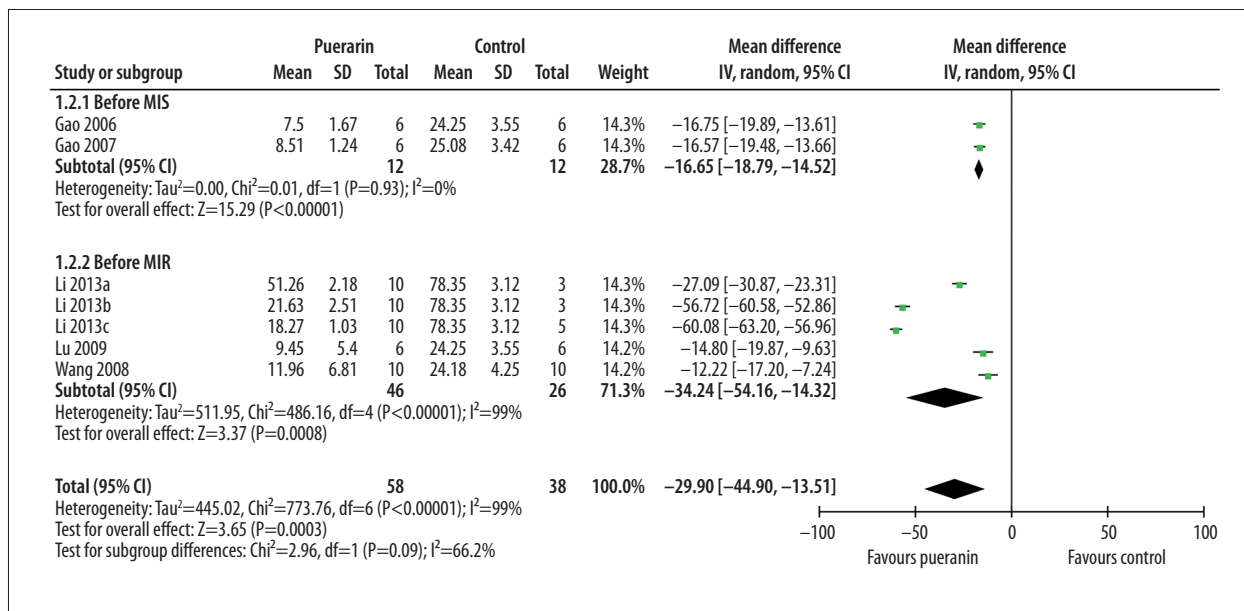

Figure 3. The effect of puerarin on size of myocardial infarction (MIN).

artery. A total of 234 rats were included, including 140 in experimental group and 94 in control group. Three studies administrated puerarin before myocardial ischemia [14–16] and four studies administrated puerarin before myocardial ischemia reperfusion [17,19–21]. One studies had separate experimental arms administrated puerarin before myocardial ischemia or before myocardial ischemia reperfusion [18]. Size of MIS and size of MIN, CK, MDA and SOD were the mostly reported outcome data.

Puerarin significantly reduced size of MIN but not MIS

Three studies reported the effect of puerarin on the size of MIS and five studies reported the outcome of MIN. Generally,

puerarin had no effect on size of MIS (mean difference: −1.40, 95%CI: −3.34 to 0.55, p=0.16), no matter administrated before MIS (mean difference: −0.91, 95%CI: −3.10 to 1.27, p=0.41) or before MIR (mean difference: −3.24, 95%CI: −7.51 to 1.03, p=0.14) (Figure 2). However, puerarin could effectively reduce the size of MIN (mean difference: −29.20, 95%CI: −44.90 to −13.51, p=0.0003), no matter administrated before MIS (mean difference: −16.65, 95%CI: −18.79 to −14.52, p<0.00001) or before MIR (mean difference: −34.24, 95%CI: −54.16 to −14.32, p=0.0008) (Figure 3).
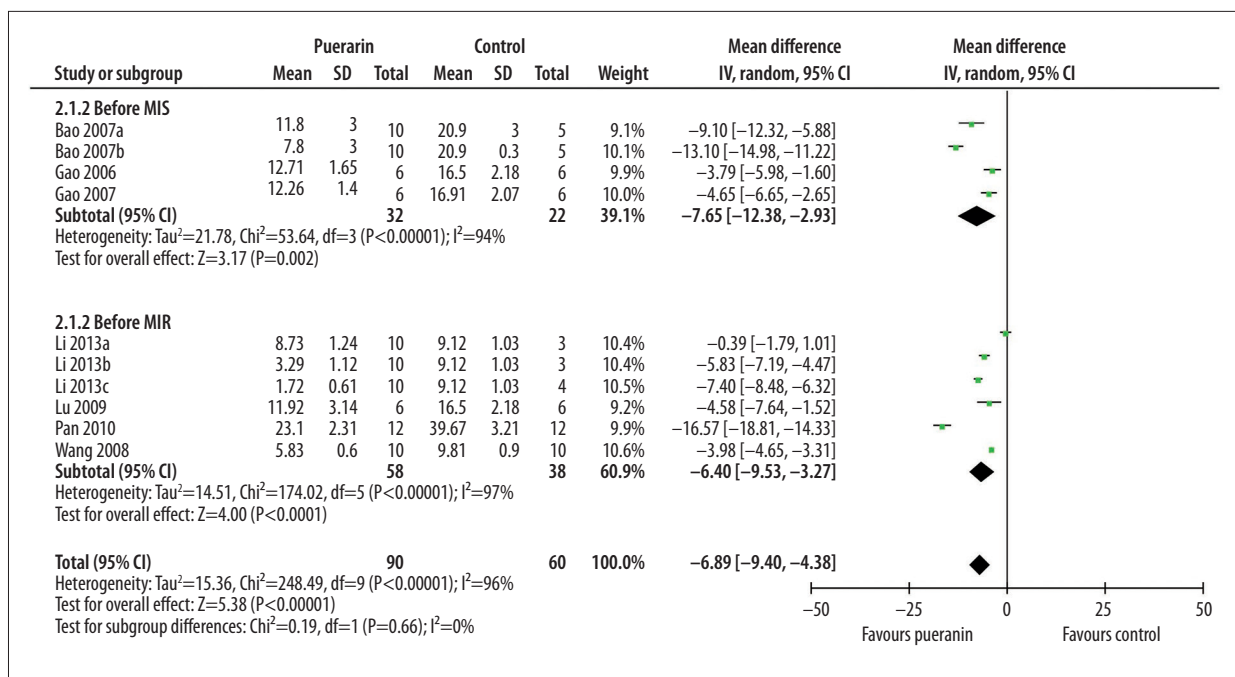
Figure 4. The effect of puerarin on serum CK.
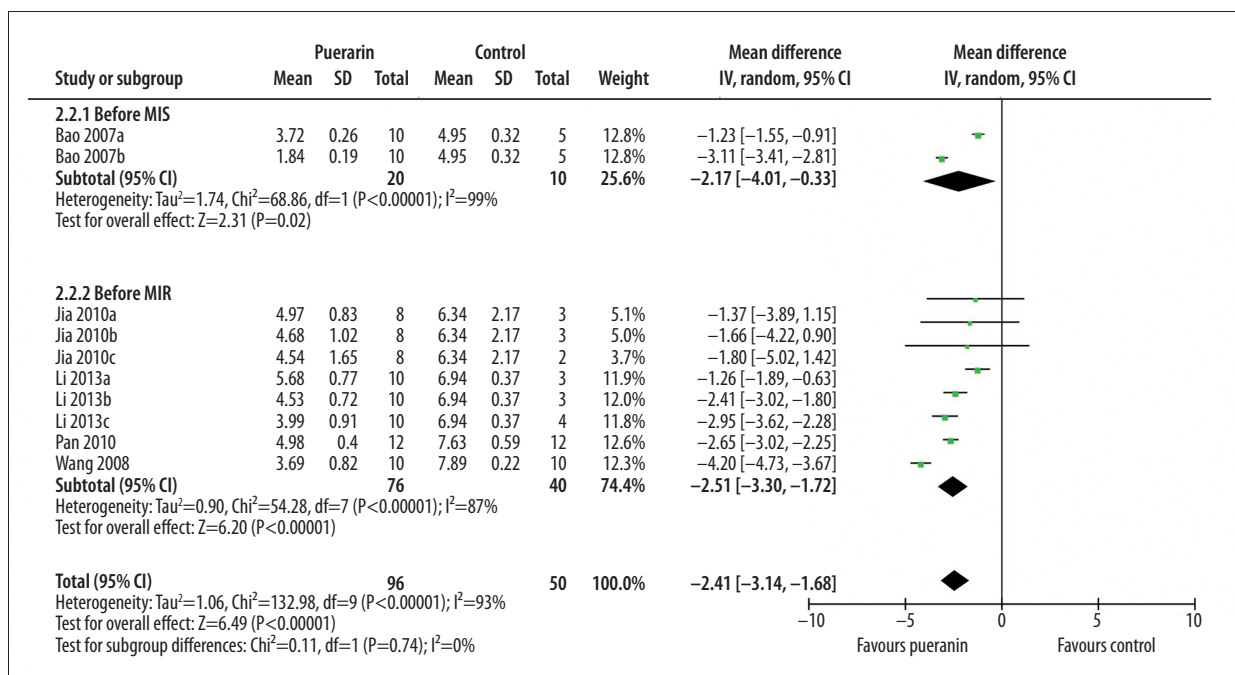
Figure 5. The effect of puerarin on serum MDA.

Puerarin significantly reduced serum CK

Five studies reported the effect of puerarin on serum CK. Puerarin could significantly reduce the level of serum CK (mean difference: −6.89, 95%CI: −9.40 to −4.38, p=0.00001). Puerarin given before MIS (mean difference: −7.65, 95%CI: −12.38 to −2.93, p=0.002) or before MIR (mean difference: −6.40, 95%CI: −9.53 to −3.27, p<0.0001) both had significant effect (Figure 4).

Puerarin significantly reduced serum MDA

Five studies reported the effect of puerarin on serum MDA. Puerarin could significantly reduce the level of serum MDA

Study or subgroup	Control			Puerarin			Weight	Mean difference IV, random, 95% CI	Mean difference IV, random, 95% CI
	Mean	SD	Total	Mean	SD	Total			
2.3.1 Before MIS									
Bao 2007a	641	66	10	511	67	5	7.2%	130.00 [58.43, 201.57]	
Bao 2007b	766	81	10	511	67	5	6.6%	255.00 [177.74, 332.26]	
Subtotal (95% CI)			20			10	13.8%	191.62 [69.13, 314.10]	
Heterogeneity: Tau²=6368.85, Chi²=5.41, df=1 (P=0.02); I²=82%									
Test for overall effect: Z=3.07 (P=0.002)									
2.3.2 Before MIR									
Jia 2010a	222.56	30.59	8	192.74	20.23	3	13.1%	29.82 [−1.38, 61.02]	
Jia 2010b	231.24	25.93	8	192.74	20.23	3	13.4%	38.50 [9.40, 67.60]	
Jia 2010c	234.42	21.48	8	192.74	20.23	2	13.0%	41.68 [9.94, 73.42]	
Li 2013a	200.11	10.14	10	183.23	9.74	3	15.5%	16.88 [4.19, 29.57]	
Li 2013b	241.25	11.04	10	183.23	9.74	3	15.5%	58.02 [45.05, 70.99]	
Li 2013c	257.81	8.73	10	183.23	9.74	4	15.7%	74.58 [63.61, 85.55]	
Subtotal (95% CI)			54			18	86.2%	44.16 [21.37, 66.65]	
Heterogeneity: Tau²=661.24, Chi²=49.39, df=5 (P<0.00001); I²=90%									
Test for overall effect: Z=3.85 (P=0.0001)									
Total (95% CI)			74			28	100.0%	63.97 [38.19, 89.75]	
Heterogeneity: Tau²=1071.20, Chi²=80.78, df=7 (P<0.00001); I²=91%									
Test for overall effect: Z=4.86 (P<0.00001)									
Test for subgroup differences: Chi²=5.39, df=1 (P=0.02); I²=81.4%									

−200 −100 0 100 200
Favours control   Favours puerarin

Figure 6. The effect of puerarin on serum SOD activity.

(mean difference: −2.41, 95%CI: −3.14 to −1.68, p<0.00001). Puerarin given before MIS (mean difference: −2.17, 95%CI: −4.01 to −0.33, p=0.02) or before MIR (mean difference: −2.51, 95%CI: −3.30 to −1.72, p<0.00001) both had significant effect (Figure 5).

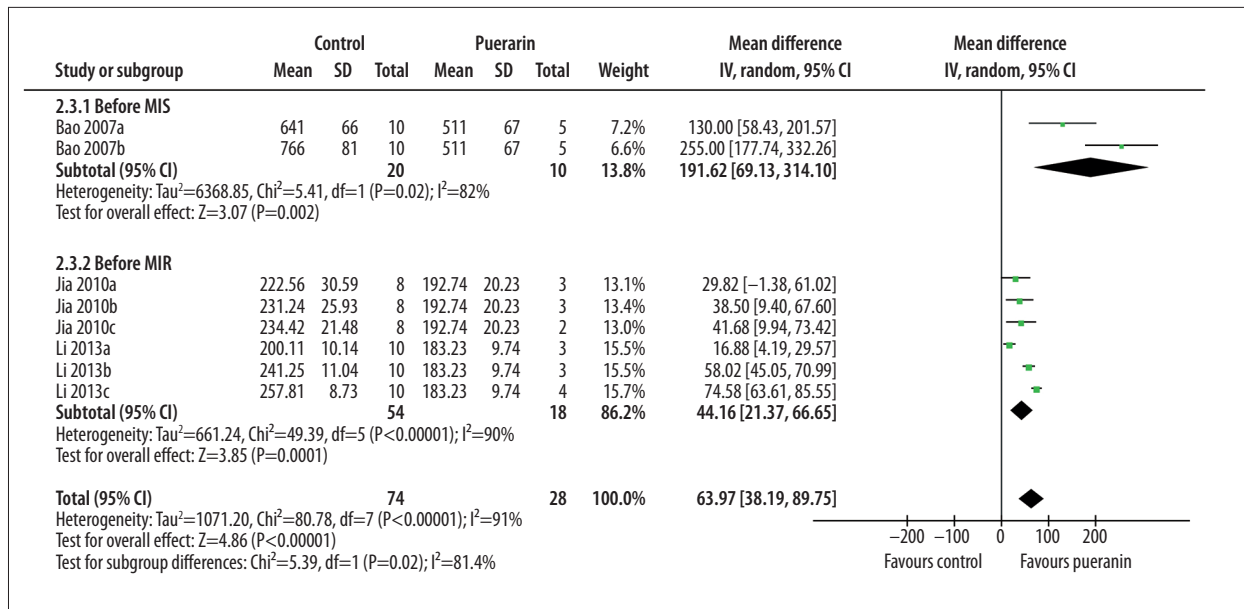## Puerarin significantly increased serum SOD activity

Five studies reported the effect of puerarin on serum SOD. Puerarin could significantly increase the level of serum SOD activity (mean difference: 63.97, 95%CI: 38.19 to 89.75, p<0.00001). Puerarin given before MIS (mean difference: 191.62, 95%CI: 69.13 to 314.10, p=0.002) or before MIR (mean difference: 44.16, 95%CI: 21.67 to 66.65, p=0.0001) both had significant effect (Figure 6).

Discussion

In China, puerarin has been used to treat patients with coronary artery diseases. A series of studies explored its effect in clinical use and found this agent could improve signs and symptoms of unstable angina and also attenuate ischemia-reperfusion injury [10,22]. In this study, we pooled previous studies that evaluated the effects of puerarin in MIRI based on rat models. Generally, we found administration of puerarin before MIS or before MIR could both effectively reduce the size of MIN after MIR, suggesting puerarin does have a protective effect for myocardial tissues.

Previous mechanism studies showed that the therapeutic effects of puerarin might be achieved through increasing SOD

activity, upregulating Bcl-2, improving the myocardial ultramicrostructure, activating the mitochondrial ATP-sensitive potassium channel, inhibiting myocardial apoptosis, reducing Bax expression, inhibiting the production of proinflammatory cytokines, refraining the calcium overload, and inhibiting mitochondrial permeability transition pore opening [23]. However, the mechanism studies are largely based on measurement of typical serum or tissue indicators in animal models. Due to the small number of animals in individual studies, their statistically power is relatively weak. In fact, the molecular mechanism of myocardial infarction reperfusion injury is complex. It is necessary to assess the outcome indicators with a large sample base. Generally, this injury is largely related to free radicals and other reactive oxygen species. During the MIRI, a large amount the radicals and reactive oxygen species are generated, leading to peroxidation of the lipids of cell membranes. MDA formed by the breakdown of lipid peroxides can result in protein conjugation and further damage membrane structure and functions [24]. Thus, the level of MDA can indicate the level of lipid peroxidation and thus indirectly reflect the degree of cell damage. CK is released from damaged myocardial cells and is considered a cardiac-specific marker of acute myocardial infarction [25]. SOD can catalyze the dismutation of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). If the activity of this enzyme decreased, oxygen-free radicals are accumulated and thereby resulting in higher level of damage. Therefore, SOD activity can reflect antioxidant function *in vivo* and is an important antioxidant protecting cells from damage due to superoxide. In this study, we observed that compared with I/R group, puerarin+IR group had significantly lower serum CK and MDA, but had higher SOD, suggesting puerarin can induce

higher level of serum of SOD and thus partly offset the negative effects of MIRI.

This study also has several limitations. Firstly, the outcome indicators were not consistent in original studies. Therefore, some important myocardial functional indicators, such as LDH, NO, and GSH were only reported in 1 or 2 original studies; therefore, it would be meaningless to pool these data. Secondly, since the agent is extracted from a traditional Chinese medicine, the original studies were all published in Chinese. The methodological quality of the included studies was generally poor. Most of the original studies had higher risk of bias in blinding assessment of outcome, which means the effect of

puerarin was likely to be overestimated. This also might be a reason for the high heterogeneity of the results. Therefore, to further confirm the therapeutic effects of puerarin in MIRI, more animal studies and randomized controlled clinical trials are required.

Conclusions

Puerarin might have a protective effect for myocardial tissues during MIRI through increasing SOD and decreasing MDA and CK. However, more animal studies and randomized controlled clinical trials are needed to confirm these results.

References:

1. Sanada S, Komuro I, Kitakaze M: Pathophysiology of myocardial reperfusion injury: preconditioning, postconditioning, and translational aspects of protective measures. Am J Physiol Heart Circ Physiol, 2011; 301(5): H1723–41

2. Sirotkovic-Skerlev M, Plestina S, Bilic I, Kovac Z: [Pathophysiology of ischaemia-reperfusion injury]. Lijecnicki vjesnik, 2006;128(3–4): 87–95 [in Croatian]

3. Luo Y, Pan YZ, Zeng C et al: Altered serum creatine kinase level and cardiac function in ischemia-reperfusion injury during percutaneous coronary intervention. Med Sci Monit, 2011; 17(9): CR474–79

4. Yellon DM, Baxter GF: Protecting the ischaemic and reperfused myocardium in acute myocardial infarction: distant dream or near reality? Heart, 2000; 83(4): 381–87

5. Szczesny B, Modis K, Yanagi K et al: AP39, a novel mitochondria-targeted hydrogen sulfide donor, stimulates cellular bioenergetics, exerts cytoprotective effects and protects against the loss of mitochondrial DNA integrity in oxidatively stressed endothelial cells *in vitro*. Nitric Oxide, 2014; 41: 120–30

6. Wang L, Li G, Wang Z et al: Elevated expression of C3G protein in the peri-infarct myocardium of rats. Med Sci Monit Basic Res, 2013; 19: 1–5

7. Luo CF, Hou N, Tian J et al: Metabolic profile of puerarin in rats after intragastric administration of puerarin solid lipid nanoparticles. Int J Nanomedicine, 2013; 8: 933–40

8. Hsu FL, Liu IM, Kuo DH et al: Antihyperglycemic effect of puerarin in streptozotocin-induced diabetic rats. J Nat Prod, 2003; 66(6): 788–92

9. Gao L, Ji X, Song J et al: Puerarin protects against ischemic brain injury in a rat model of transient focal ischemia. Neurol Res, 2009; 31(4): 402–6

10. Zhang S, Chen S, Shen Y et al: Puerarin induces angiogenesis in myocardium of rat with myocardial infarction. Biol Pharm Bull, 2006; 29(5): 945–50

11. Xu ME, Xiao SZ, Sun YH et al: The study of anti-metabolic syndrome effect of puerarin *in vitro*. Life Sci, 2005; 77(25): 3183–96

12. Yan LP, Chan SW, Chan AS et al: Puerarin decreases serum total cholesterol and enhances thoracic aorta endothelial nitric oxide synthase expression in diet-induced hypercholesterolemic rats. Life Sci, 2006; 79(4): 324–30

13. Han RM, Tian YX, Becker EM et al: Puerarin and conjugate bases as radical scavengers and antioxidants: molecular mechanism and synergism with beta-carotene. J Agric Food Chem, 2007; 55(6): 2384–91

14. Boa X, Li Z, Qin XT: [Protective effect of puerarin preconditioning on myocardial ischemia-reperfusion injury in isolated rat hearts.] Journal of Clinical Medicine in Practice, 2007; 6(11): 15–18 [in Chinese]

15. Gao X, Li T, Gao Q et al: [The protective efect of puerarin preconditioning against myocardial ischemia-reperfusion injury in rats.] Journal Of Guiyang Medical College, 2006; 31(6): 523–26 [in Chinese]

16. Gao X, Gao Q, Li T, Lu DQ: [The mechanism of protective effect of puerarin on myocardial ischemic reperfusion injury in rats.] Journal of Guiyang Medical College, 2007; 32(1): 37–41 [in Chinese]

17. Wang J, Shang L, Cui M, Li M: [Experimental study of protective effects and mechanism of pueraria on myocardial ischemia reperfusion injury in rats.] Journal of Henan University of Chinesemedicine, 2008; 23(134): 32–34 [in Chinese]

18. Lu DQ, Gao X, Li BL: [Protective efect of puerarin preconditioning on myocardial against ischemia-reperfusion injury in Rat.] Journal of Guiyang Medical College, 2009; 34(5): 501–7

19. Jia C, Qin Y, Ren B: [Puerarin protects heart against oxidative stress induced by acute myocardial ischemia-reperfusion injury in rats.] Journal of Liaoning University Of TCM, 2010; 12: 230–32 [in Chinese]

20. Pan S, Chen W: The protective effect of puerarin over myocardial ischemia reperfusion injury in rat model. Chinese Journal of Gerontology, 2010; 30: 332–34 [in Chinese]

21. Li J, Huang K, Wang Y, Shi B: Effect of puerarin on myocardial ischemia-reperfusion injury in rat cardiac enzymes. Chinese Journal of Lab Diagnosis, 2013; 17: 244–46 [in Chinese]

22. Xie RQ, Du J, Hao YM: [Myocardial protection and mechanism of Puerarin Injection on patients of coronary heart disease with ischemia/reperfusion]. Chinese journal of Integrated Traditional and Western Medicine, 2003; 23(12): 895–97 [in Chinese]

23. Feng ZQ, Wang YY, Guo ZR et al: The synthesis of puerarin derivatives and their protective effect on the myocardial ischemia and reperfusion injury. J Asian Nat Prod Res, 2010; 12(10): 843–50

24. Wu L, Qiao H, Li Y, Li L: Protective roles of puerarin and Danshensu on acute ischemic myocardial injury in rats. Phytomedicine, 2007; 14(10): 652–58

25. Zhang Z, Lam TN, Zuo Z: Radix Puerariae: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol, 2013; 53(8): 787–811