Beneficial changes in prostacyclin and thromboxane A₂ by ginsenosides in myocardial infarction and reperfusion injury of dogs

FANG Yun-xiang, SHEN Na, CHEN Xio
(Dept of Pharmacology, Human Medical College, Chiangsha 410088)

ABSTRACT The effect of ginsenosides on creatine phosphate kinase (CPK), prostacyclin (PGI₁) and thromboxane A₂ (TXA₂) levels in coronary venous blood was studied on experimental myocardial infarction (MI) in anesthetized dogs. Stable metabolites of PGI₁ (6-keto-PGF₁α) and TXA₂ (TXB₂), were determined by RIA. In control group, 1 h occlusion of LAD and 1 h reperfusion brought about an increase of CPK and a decrease of metabolites of PGI₁/TXA₂ ratio by either increase of TXA₂ or and decrease of PGI₁. Ginsenosides 30 mg/kg iv decreased CPK and TXB₂, increased 6-keto-PGF₁α, hence increased metabolites of PGI₁/TXA₂, ratio after both coronary occlusion and reperfusion. The result indicates that ginsenosides possess protective effects on myocardial ischemia and reperfusion injury of dogs. The beneficial effect on PGI₁/TXA₂ seems to contribute to its myocardial protective action.

KEY WORDS ginsenosides: myocardial infarction: prostaglandins: thromboxane A₂: reperfusion of coronary vessels

We reported previously that ginsenosides
protected experimental acute myocardial ischemia in rabbit and rat(14,15). The possible mechanisms of protective effect were attributed to the beneficial hemodynamic and metabolic action on FFA and carbohydrate(14,15). Since some drugs effective in MI can also ameliorate reperfusion injury(16), we are prompted to investigate whether ginsenosides protect against reperfusion injury. Since the vascular effects of ginsenosides in vitro may be mediated by interaction with endogenous vasoactive substances, possibly prostaglandins(17), we aimed at the possible effects of ginsenosides on most active prostanoids to the cardiovascular system—PGF2 and TXA2, so as to get better understanding on the mechanism of myocardial protective action of ginsenosides.

MATERIALS AND METHODS

Experiment procedure Mongrel dogs of either sex, weighing 11.6 ± SD 1.1 kg, were anesthetized with sodium pentobarbital 30 mg/kg (v). Trachea was intubated for artificial respiration and femoral vein was catheterized for medication. Thoracotomy was performed in the 5th intercostal space and the heart was exposed. Cardiac catheter was inserted into the coronary sinus via right external jugular vein for blood sampling. The left anterior descending coronary artery (LAD) was isolated and a tie was passed at the upper one third so as to produce ischemic zones of uniform size approximately two-thirds of the anterior surface of left ventricle. A reversible knot was tied on occlusion, and was released on reperfusion. Biochemical assays 15 ml of coronary sinus blood were withdrawn for assay of CPK and prostanoids. CPK was estimated photometrically(18). 6-Keto-PGF1α was measured by RIA(19). Briefly, 5 ml blood were withdrawn into a syringe containing 0.2 ml indomethacin-heparin-saline solution, to prevent ex vivo generation of prostanoids and coagulation. Plasma was secured by centrifugation at 3,000 rpm for 15 min. The supernatant was aliquoted to 3.5-4.0 with 1 N HCl and [7H]-6-keto-PGF1α was added. 5 ml petroleum ether were added for extraction. After centrifugation at 3,000 rpm for 5 min the precipitate preserved for [7H]-6-keto-PGF1α assay by liquid scintillation. TXB2 was measured by RIA(15). In addition to indo- methacin-heparin-saline solution, the syringe for withdrawing blood was pretreated with 5% silica oil to prevent ex vivo release of TXA2 from platelets. Plasma was secured by centrifugation 5,000 g for 15 min at 4°C. Adjusting pH to 3.5-4.0 with 1 N HCl and adding redistilled ethyl acetate 5 ml for extraction twice. Supernatants were combined and dried in a vacuum pump. [7H]-TXB2 was added and radioactivity was measured by liquid scintillation.

Experimental protocol After thoracotomy but before occlusion of LAD, blood was withdrawn from coronary sinus for assays of CPK and prostanoids. Then ginsenosides was injected 20 mg/kg iv followed by 10 mg/kg iv infusion at a rate of 1 mg/ml/min in treated group. In control group, equivalent amount of normal saline was iv injected and infused. Immediately after iv injection of ginsenosides, during iv infusion. LAD was occluded for 1 h and released the tie to reperfuse heart for another 1 h. At the end of 1 h ischemia reperfusion blood samples were withdrawn from coronary sinus.

Experimental design and statistics 18 dogs were randomly divided into control and ginsenoside group. Group comparisons by t test were calculated to estimate the differences between changes at the same period. Chemicals Ginsenosides were extracted from Panax ginseng CA Meyer by Shibata
method. The kit for CPK was purchased from Sigma (USA). The RIA kits for 6-keto-PGF\(_1\alpha\) and TXB\(_2\) were supplied by the Institute of Basic Medicine of the Chinese Academy of Medical Sciences and the General Hospital of the Chinese Liberation Army, respectively.

**RESULTS**

Effect of ginsenosides on CPK in coronary venous blood after ligation of LAD and reperfusion. Coronary venous blood CPK increased significantly 1 h after occlusion and rose further after 1 h reperfusion (Fig 1). Ginsenosides blunted the CPK increase after coronary occlusion (p<0.05) and after reperfusion (p<0.01). The results signify that ginsenosides exert protective effect on acute myocardial ischemia as well as on reperfusion injury.

![Figure 1](image1.png)

**Fig 1.** Effect of ginsenosides on CPK in coronary venous blood after ligation of LAD and reperfusion. Before ligation (B), 1 h after ligation (L), 1 h after reperfusion (R). n=9. ** p<0.05 *** p<0.01

Effect of ginsenosides on 6-keto-PGF\(_1\alpha\) in coronary venous blood after ligation LAD and reperfusion. After occlusion of LAD, stable metabolite of PGI\(_2\) was increased in both group, slightly higher in ginsenosides group but statistically non significant. However, after 1 h reperfusion, it increased continuously in ginsenosides group and lowered in control group (Fig 2). The difference was highly significant.

![Figure 2](image2.png)

**Fig 2.** Effect of ginsenosides on 6-keto-PGF\(_1\alpha\) in coronary venous blood after ligation of LAD and reperfusion. Before ligation (B), 1 h after ligation (L), 1 h after reperfusion (R). n=9. *** p<0.01

Effect of ginsenosides on TXB\(_2\) in coronary venous blood after ligation of LAD and reperfusion. After ligation of LAD in dogs, TXB\(_2\) were increased in both group, but more (p<0.05) in the control group than in ginsenosides group. TXB\(_2\) in the control group increased continuously after reperfusion, while TXB\(_2\) tended to decrease in ginsenosides group (Fig 3). The difference being highly significant.

![Figure 3](image3.png)

**Fig 3.** Effect of ginsenosides on TXB\(_2\) in coronary venous blood after ligation of LAD and reperfusion. Before ligation (B), 1 h after ligation (L), 1 h after reperfusion (R). n=9. ** p<0.05 *** p<0.01
Effect of ginsenosides on the ratio metabolites of PG1/TXA3 in coronary venous blood during ischemia and reperfusion

In the control group, the ratio reduced from normally 7.10 to 3.43 in ischemia and further reduced to 2.32 after reperfusion. In ginsenosides group, the decrease of ratio was blunted from normally 7.10 to 4.78 in ischemia and returned to 6.23 after reperfusion. The results indicate both myocardial ischemia and reperfusion alter the balance between PG1 and TXA3, and favor TXA3, increase, thus aggravate myocardial ischemia. Though the ratio PG1/TXA3 after LAD ligation decreases 32% in the ginsenosides group, it is still significantly higher than in the control group which decreases 24%. The ratio turns to increase again after reperfusion in ginsenosides group (remaining only 113% less than normal) in contrast to the control group (67% less than normal) \( p < 0.01 \).

**DISCUSSIONS**

The present work has confirmed the protective effect of ginsenosides on acute myocardial infarction in dogs just as previously observed in rats & rabbits\(^{11,12}\). We have demonstrated for the first time a protective effect of ginsenosides on myocardial reperfusion injury with beneficial changes of prostanooids.

It has been well documented that under certain conditions, coronary reperfusion can aggravate myocardial ischemia. The underlying mechanism of reperfusion injury has not been elucidated yet. However, changes of PG1 and TXA3 seem to play a role at least in reperfusion-induced arrhythmia\(^{14,15}\). PG1 is the most potent naturally-occurring antiaggregatory agent as well as a potent vasodilator. The directly opposing vasoactive and platelet active properties of TXA3, and PG1 constitute a ratio of balance, which has important implications in physiological and pathological conditions. The imbalance caused by either reduction of PG1 or/and increased production of TXA3, brings about a lowering of PG1/TXA3 ratio during myocardial ischemia and reperfusion, which denotes a decrease in vasodilatory and antiplatelet action, thus exacerbating myocardial ischemia. An imbalance in the ratio provides an explanation to the pathological changes in myocardial infarction. New approaches to treatment of myocardial ischemia are being sought by developing drugs that tilt the balance in favor of prostacyclin either by inhibiting TXA3, synthetase, or protecting PG1 synthetase or increasing prostacyclin release\(^{11}\).

Ginsenosides prevented the reduction of PG1/TXA3 ratio, reversed the detrimental changes of prostanooids, and certainly contributed to the protective action on myocardial ischemia and reperfusion damage. The mechanism of antithrombotic action of PG1 on platelets was related to its potent stimulant effect on platelet adenylyl cyclase, resulting in an elevation of cAMP level, which in turn, promotes calcium removal and consequently reduces the cytoplasmic calcium level\(^{11}\).

In contrast to PG1, TXA3, can reduce platelet intracellular cAMP and cytoplasmic calcium level\(^{11}\). It has been shown that ginsenosides can increase the cAMP/cGMP ratio in hypoxic myocardium of mice\(^{15}\). This may be attributed to the enhanced release of PG1 or reduced synthesis of TXA3, which contribute to the beneficial effect of ginsenosides on myocardial ischemia and reperfusion.

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人参皂苷对犬心肌梗塞和再灌注损伤及其前列环素和血栓素A2变化的有益作用

方公祥，沈 乃，陈 修（湖南医学院病理教研室，长沙 410008）

摘要 通过大鼠心肌梗塞再灌注心肌损伤模型，观察了用不同浓度的6-keto-PGF1α的药物对心肌梗塞和再灌注后红细胞膜磷脂过氧化反应的影响，结果表明，6-keto-PGF1α能显著降低心肌梗塞和再灌注后红细胞膜磷脂过氧化水平。同时，6-keto-PGF1α能显著降低心肌梗塞和再灌注后红细胞膜磷脂过氧化水平，显示了6-keto-PGF1α对心肌梗塞和再灌注损伤的保护作用。

关键词 人参皂苷，心肌梗死，再灌注损伤，前列环素，血栓素A2