

## Beneficial changes in prostacyclin and thromboxane A<sub>2</sub> by ginsenosides in myocardial infarction and reperfusion injury of dogs<sup>1</sup>

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**ABSTRACT** The effect of ginsenosides on creatine phosphate kinase (CPK), prostacyclin (PGI<sub>2</sub>) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) levels in coronary venous blood was studied on experimental myocardial infarction (MI) in anesthetized dogs. Stable metabolites of PGI<sub>2</sub> (6-keto-PGF<sub>1α</sub>) and TXA<sub>2</sub> (TXB<sub>2</sub>), 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 PGI<sub>2</sub>/TXA<sub>2</sub> ratio by either increase of

TXA<sub>2</sub> or/and decrease of PGI<sub>2</sub>. Ginsenosides 30 mg/kg iv decreased CPK and TXB<sub>2</sub>, increased 6-keto-PGF<sub>1α</sub>, hence increased metabolites of PGI<sub>2</sub>/TXA<sub>2</sub> 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 PGI<sub>2</sub>/TXA<sub>2</sub> seems to contribute to its myocardial protective action.

**KEY WORDS** ginsen; saponins; myocardial infarction; prostaglandins X; thromboxane A<sub>2</sub>; reperfusion of coronary vessels

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We reported previously that ginsenosides

protected experimental acute myocardial ischemia in rabbits and rats<sup>(1,2)</sup>. The possible mechanisms of protective effect were attributed to the beneficial hemodynamic and metabolic action on FFA and carbohydrate<sup>(2,4,5)</sup>. Since some drugs effective in MI can also ameliorate reperfusion injury<sup>(6)</sup>, 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 substance, possibly prostaglandins<sup>(7)</sup>, we aimed at the possible effects of ginsenosides on most active prostanoids to the cardiovascular system—PGI<sub>2</sub> and TXA<sub>2</sub>, 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  $11.6 \pm \text{SD } 1.1 \text{ kg}$ , were anesthetized with sodium pentobarbital 30 mg/kg iv. 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<sup>(8)</sup>. 6-Keto-PGF<sub>1 $\alpha$</sub>  was measured by RIA<sup>(9)</sup>. Briefly, 5 ml blood were withdrawn in 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 10 min. The pH was adjusted to 3.5-4.0 with 1 N HCl and [<sup>3</sup>H]6-keto-PGF<sub>1 $\alpha$</sub>  was added. 5 ml petroleum ether were added for extraction. After centrifugation at 3,000 rpm for 5 min the precipitate preserved was for 6-keto-PGF<sub>1 $\alpha$</sub>  assay by liquid scintillation. TXB<sub>2</sub> was measured by RIA<sup>(10)</sup>. In addition to indomethacin-heparin-saline solution, the syringe for withdrawing blood was pretreated with 5% silica oil to prevent *ex vivo* release of TXA<sub>2</sub> 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; [<sup>3</sup>H]-TXB<sub>2</sub> 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 ginsenosides 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<sub>1α</sub> and TXB<sub>2</sub> 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 rised 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.

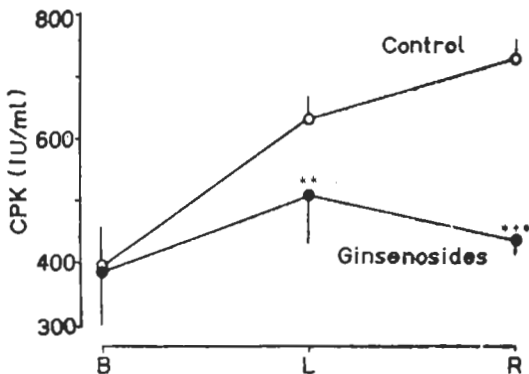


Fig 1. Effect of ginsenosides on CPK in coronary venous Blood after ligation of LAD and reperfusion. Before ligation (B), 1 h after ligation (L), 1 h after reperfusion (R).  $n = 9$ . \*\*  $p < 0.05$  \*\*\* $p < 0.01$

**Effect of ginsenosides on 6-keto-PGF<sub>1α</sub> in coronary venous blood after ligation LAD and reperfusion** After occlusion of LAD, stable metabolite of PGI<sub>2</sub> 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.

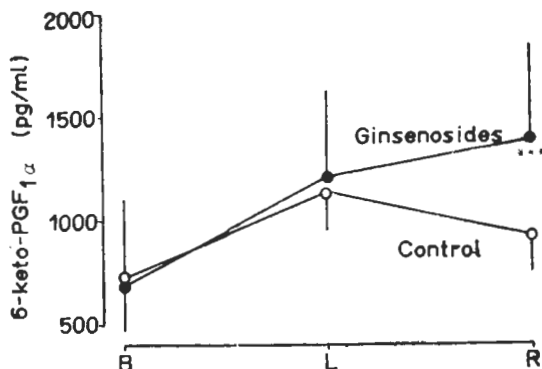


Fig 2. Effect of ginsenosides on 6-keto-PGF<sub>1α</sub> in coronary venous blood after ligation of LAD and reperfusion. Before ligation (B), 1 h after ligation (L), 1 h after reperfusion (R).  $n = 9$ . \*\*\* $p < 0.01$

**Effect of ginsenosides on TXB<sub>2</sub> in coronary venous blood after ligation of LAD and reperfusion** After ligation of LAD in dogs, TXB<sub>2</sub> were increased in both group, but more ( $p < 0.05$ ) in the control group than in ginsenosides group. TXB<sub>2</sub> in the control group increased continuously after reperfusion, while TXB<sub>2</sub> tended to decrease in ginsenosides group (Fig 3), the difference being highly significant.

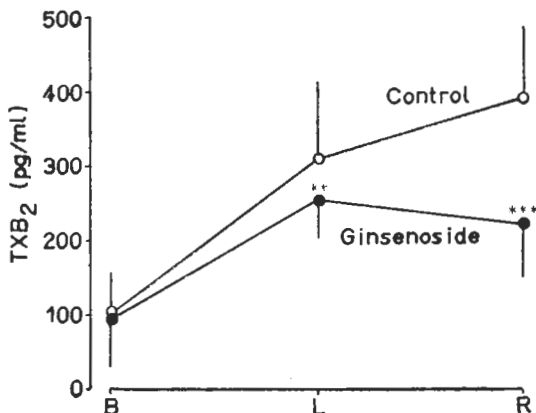


Fig 3. Effect of ginsenosides on TXB<sub>2</sub> in coronary venous blood after ligation of LAD and reperfusion. Before ligation (B), 1 h after ligation (L), 1 h after reperfusion (R).  $n = 9$ . \*\*  $p < 0.05$  \*\*\*  $p < 0.01$

### Effect of ginsenosides on the ratio metabolites of PGI<sub>2</sub>/TXA<sub>2</sub> 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 7.00 to 4.78 in ischemia and returned to 6.23 after reperfusion. The results indicate both myocardial ischemia and reperfusion alter the balance between PGI<sub>2</sub> and TXA<sub>2</sub> and favor TXA<sub>2</sub> increase, thus aggravate myocardial ischemia. Though the ratio PGI<sub>2</sub>/TXA<sub>2</sub> after LAD ligation decreases 32% in the ginsenosides group, it is still significantly higher than that in the control group which decreases 52%. The ratio turns to increase again after reperfusion in ginsenosides group (remains only 11% 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<sup>(1,2)</sup>. We have demonstrated for the first time a protective effect of ginsenosides on myocardial reperfusion injury with beneficial changes of prostanoids.

It has been well documented that under certain conditions, coronary reperfusion can aggravate myocardial ischemia. The underlying mechanisms of reperfusion injury has not been elucidated yet. However, changes of PGI<sub>2</sub> and TXA<sub>2</sub> seem to play a role at least in reperfusion-induced arrhythmia<sup>(11)</sup>. PGI<sub>2</sub> is the most potent naturally-occurring antiaggregatory agent as well as a potent vasodilator. The directly opposing vasoactive and platelet active properties of TXA<sub>2</sub> and PGI<sub>2</sub> constitute a ratio of balance, which has important implications in physiological and pathological

conditions. The imbalance caused by either reduction of PGI<sub>2</sub> or/and increased production of TXA<sub>2</sub> brings about a lowering of PGI<sub>2</sub>/TXA<sub>2</sub> 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 TXA<sub>2</sub> synthetase, or protecting PGI<sub>2</sub> synthetase or increasing prostacyclin release<sup>(12)</sup>.

Ginsenosides prevented the reduction of PGI<sub>2</sub>/TXA<sub>2</sub> ratio, reversed the detrimental changes of prostanoids, and certainly contributed to the protective action on myocardial ischemia and reperfusion damage.

The mechanism of antiaggregatory action of PGI<sub>2</sub> on platelets was related to its potent stimulant effect on platelet adenylate cyclase, resulting in an elevation of cAMP level, which in turn, promotes calcium removal and consequently reduces the cytoplasmic calcium level<sup>(13)</sup>.

In contrast to PGI<sub>2</sub>, TXA<sub>2</sub> can reduce platelet intracellular cAMP and cytoplasmic calcium level<sup>(13)</sup>. It has been shown that ginsenosides can increase the cAMP/cGMP ratio in hypoxic myocardium of mice<sup>(5)</sup>. This may be attributed to the enhanced release of PGI<sub>2</sub> or reduced synthesis of TXA<sub>2</sub>, which contribute to the beneficial effect of ginsenosides on myocardial ischemia and reperfusion.

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## 人参皂甙对犬心肌梗塞和再灌注损伤及其前列环素和血栓素 A<sub>2</sub> 变化的有益作用

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**提要** 麻醉犬结扎 LAD 形成心肌梗塞。冠状窦插管取血, 放射免疫法测定 PGI<sub>2</sub> 和 TXA<sub>2</sub> 的代谢物 6-keto-PGF<sub>1α</sub> 和 TXB<sub>2</sub>。对照组(n=9)结扎冠脉和重灌注 1 h, CPK 和 TXA<sub>2</sub> 显著增加, PGI<sub>2</sub> 下降, 因而 PGI<sub>2</sub>/TXA<sub>2</sub> 比值下降。iv 人参总皂甙 30 mg/kg 能降低心肌梗塞和重灌注犬的 CPK 和 TXB<sub>2</sub> 值, 增加

6-keto-PGF<sub>1α</sub> 值, 因而增加 PGI<sub>2</sub>/TXA<sub>2</sub> 比值。结果显示人参总皂甙有保护犬心肌缺血和重灌注损伤的作用。

**关键词** 人参皂甙; 心肌梗塞; 再灌注损伤; 前列环素; 血栓素 A<sub>2</sub>