

三七皂甙对大鼠实验性心肌缺血再灌注损伤的保护作用

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Protective effects of *Panax notoginseng* saponins on experimental myocardial injury induced by ischemia and reperfusion in rat

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ABSTRACT Effects of total saponins of *Panax notoginseng* (PNS) and purified ginsenosides R_{b1} and R_{g1} from PNS on myocardial injury induced by cardiac ischemia and reperfusion were studied with rat hearts *in situ* and *in vitro*. In pentobarbital-anesthetized rats, PNS pretreatment (100 and 200 mg/kg) provided significant reduction in myocardial infarct size after left descending coronary artery ligation (40 min) and reperfusion (120 min) in comparison with the control. PNS 12.5 and 25 mg/L, R_{b1} 10 mg/L, and R_{g1} 10 mg/L significantly decreased cardiac CPK release, attenuated myocardial Ca²⁺ accumulation, reduced malondialdehyde (MDA) production and prevented reduction of superoxide dismutase (SOD) activity in comparison with the control in perfused isolated rat hearts with global ischemia (40 min) and reperfusion (15 min). The results show that PNS, R_{b1}, and R_{g1} prevent cardiac ischemia and the action is considered to be related to the inhibition of lipid peroxidation.

KEY WORDS ginseng; saponins; superoxide dismutase; lipid peroxides; calcium channel blockers; myocardial reperfusion injury

摘要 三七总皂甙(PNS)呈剂量依赖性地缩小在体大鼠冠状动脉结扎-再通后心肌梗塞范围,减轻离体灌流大鼠心脏低灌-复灌引起的心肌CPK释放和钙聚集,显著保护SOD活力的降低,减少MDA生成。皂甙单体R_{b1}、R_{g1}在离体鼠心与PNS作用一致。提示三七皂甙的抗脂质过氧化作用是其保护缺血再灌注心肌的一个重要方面。

Received 1988 Dec 6 Accepted 1989 May 3
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关键词 人参; 皂甙; 超氧化物歧化酶; 脂质过氧化物; 钙通道阻滞剂; 心肌再灌注损伤

三七(*Panax notoginseng*)皂甙能降低猫、兔、犬的动脉压⁽¹⁾,在离体豚鼠心房和乳头状肌呈负性变频和变力作用⁽²⁾,抑制血小板聚集⁽³⁾,并经离体血管条和心肌细胞电压钳实验证实为钙通道阻滞剂⁽⁴⁻⁶⁾。本文以钙通道阻滞剂维拉帕米(verapamil, Ver)为阳性对照药,1)观察三七皂甙对大鼠在体心肌缺血再灌注损伤的影响;2)以CPK释放和心肌钙聚集为指标,观察三七皂甙对离体大鼠心脏缺血再灌注损伤的作用;3)以超氧化物歧化酶(SOD)活力和脂质过氧化产物丙二醛(MDA)生成作为间接依据,探讨三七皂甙对氧自由基损伤心肌的影响。

MATERIALS AND RESULTS

PNS由广西梧州第三制药厂提供,薄层鉴定含7个皂甙色点。从PNS提取的人参皂甙单体R_{b1}、R_{g1}由广州市医药工业研究所植化室惠赠,薄层鉴定均为单一斑点。用生理盐水(NS)将药物配成所需浓度。

PNS对麻醉大鼠缺血再灌注心肌梗塞范围的影响 本校实验动物中心提供的SD封闭群大鼠40只,♂,体重210±SD 37g。随机分为5组。ip戊巴比妥钠45mg/kg麻醉,经胸骨旁切口纵行开胸,立即正压人工呼吸(潮气量2ml/100g,50次/min)。暴露心脏,在肺动脉圆锥左缘、平左心耳下缘经浅层心肌穿一条5/0丝线,冠脉结扎和再灌注前10min分别恒速iv NS, Ver 1.25mg/kg, PNS 50, 100, 200mg/kg(4ml/kg, 0.1ml/min)。结扎冠状动脉前降支造成心肌缺血。40min时剪开结扎线,再灌注120min,实验过程中定期监测V₁导联ECG。摘取心脏,除去心房,

将心室横切成3-4片, 置入pH 7.4的氯化硝基四氮唑蓝(NBT)溶液, 于37°C染色10 min⁽⁷⁾, 分离梗死心肌与正常心肌, 分别称重, 计算梗死心肌占全心室肌湿重的%(Tab 1)。

Tab 1. Effects of *Panax notoginseng* saponins (PNS) and verapamil on the infarct size in the ischemic and reperfused rat hearts *in situ*. $n=8$, $\bar{x}\pm SD$. * $P>0.05$, *** $P<0.01$ vs control, † $P>0.05$, †† $P<0.01$ vs verapamil.

Drug	Dose (mg/kg)	Infarct size (%)
Control	—	31.4±4.1
Verapamil	1.25	20.7±1.7***
PNS	50	28.7±2.4*††
PNS	100	23.5±3.3***†
PNS	200	21.9±1.5***†

可以看到, PNS能够缩小缺血再灌注心肌梗塞范围, 呈明显的剂量依赖性($r=-0.939$, $P<0.05$). PNS 100, 200 mg/kg与Ver 1.25 mg/kg的作用差异不显著。

三七皂甙对离体灌流大鼠心脏缺血再灌注损伤的影响 SD大鼠69只, ♂, 体重220±SD 39 g. 肝素化后迅速取心脏进行Langendorff恒压灌流, 分三期: 1) 预灌期, 20 min, 灌流液为修正Kreb's液⁽⁸⁾, 37°C, 通纯O₂, pH 7.3-7.4. 预灌5 min始人工起搏心脏, 控制心率于300 bpm, 直至实验结束. 冠脉流量7-10 ml/min. 2) 低灌期, 40或60 min, 停止恒压灌注而进行恒流灌注, 将冠脉流量控制在0.3-0.4 ml/min, 心脏温度33-34°C. 3) 再灌注期, 15 min, 心脏恢复恒压灌注. 给药组将药物加入灌流液, 配成所需浓度, 于实验全程给药。

1 心肌CPK释放 收集各组心脏再灌注第15 min冠脉流出液, 在IL Monach 761型自动生化分析仪测定CPK活力, 换算成U/(min·g·dry wt)(Tab 2)。

可见, PNS 12.5, 25 mg/L对心肌缺血60 min, 再灌注15 min时的CPK释放无显著性影响; 而将心肌缺血时间缩短至40 min,

Tab 2. Effects of PNS, ginsenoside R_{b1}, R_{g1}, and verapamil on the creatine phosphokinase (CPK) release after 15 min reperfusion following 40 or 60 min global ischemia in the rat hearts *in vitro*. $n=5-7$, $\bar{x}\pm SD$. * $P>0.05$, ** $P<0.05$, *** $P<0.01$ vs control.

Drug	Concentration (mg/L)	CPK(U/min·g dry wt)	
		40 min	60 min
Control	—	5.4±1.4	8.0±3.9
Verapamil	2 μmol/L	2.4±0.8***	2.4±0.7***
PNS	12.5	2.7±1.0***	6.7±2.8*
PNS	25	3.0±1.4***	9.9±3.1*
R _{b1}	10	3.8±1.0**	7.9±1.3*
R _{g1}	10	3.4±0.8**	—

PNS及其单体R_{b1}、R_{g1}则显著减少再灌注第15 min时的CPK释放. Ver 2 μmol/L在缺血40或60 min情况下, 都显著减少再灌注第15 min时的CPK释放。

2 心肌钙聚集⁽⁹⁾ 离体灌流大鼠心脏, 低灌40 min, 再灌注15 min后, 立即用预冷的、经732强酸性阳离子交换树脂处理的0.35 mol/L蔗糖-5 mmol/L组氨酸溶液(pH 7.4) 10 ml灌洗, 冲洗细胞外间隙钙⁽¹⁰⁾. 全心室于100°C烘干至恒重, 用3 ml浓硝酸消化48 h, 稀释10倍后, 在HITACHI 180-70型原子吸收光谱仪(波长422.7 nm)上测定钙含量, 钙含量以μmol/(g·dry wt)表示(Fig 1)。

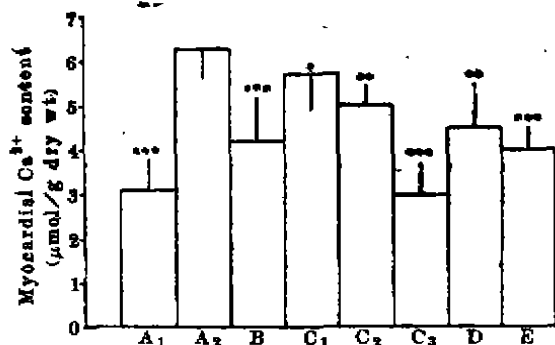


Fig 1. Effects of PNS 6.25, 12.5, 25 mg/L (C₁, C₂, C₃), R_{b1} 10 mg/L (D), R_{g1} 10 mg/L (E), and verapamil 2 μmol/L (B, $n=4$) on the myocardial Ca²⁺ accumulation in the global ischemic and reperfused rat heart. $n=6$, $\bar{x}\pm SD$. * $P>0.05$, ** $P<0.05$, *** $P<0.01$ vs control (A₂), A₁=normal ($n=5$).

缺血 40 min, 再灌注 15 min 使心肌钙含量显著升高。PNS 显著减轻缺血再灌注引起的心肌钙聚集, 并呈显著剂量依赖性 ($r = -0.995, P < 0.01$), 与 Ver $2 \mu\text{mol/L}$ 作用一致。R_{b1}、R_{r1} 也有相同作用。

三七皂甙对缺血再灌注大鼠心脏 SOD 活力和 MDA 生成的影响 SD 大鼠 42 只, ♂, 体重 $210 \pm \text{SD } 26 \text{ g}$, 随机分为 7 组, 制备 Langendorff 灌流心脏。正常对照组恒压灌注 80 min, 其余低灌 40 min 后再灌注 15 min, 分别作空白对照和给药。灌注结束后, 取左心室前壁全层心肌约 0.2 g, 制备 20% (wt/vol) 匀浆, 测定 SOD 活力和 MDA 生成。

1 心肌 SOD 活力 以 Sigma 产品 SOD (3300 U/mg 蛋白) 为标准品, 按光化学扩增法^[11]测定每克心肌组织中 SOD 活力 (Fig 2)。

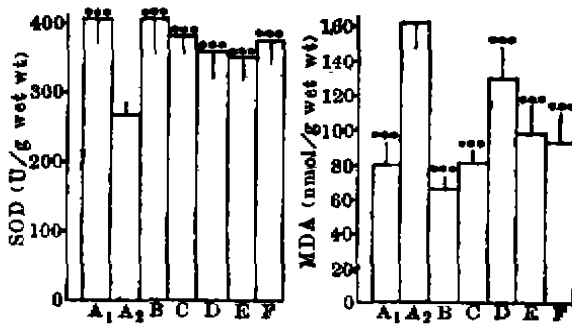


Fig 2. Effects of PNS 12.5 mg/L (D), R_{b1} 10 mg/L (E), R_{r1} 10 mg/L (F), and verapamil $2 \mu\text{mol/L}$ (B), allopurinol 1 mmol/L (C) on myocardial superoxide dismutase (SOD) activity (Left) and malondialdehyde (MDA) production (Right) in the global ischemic and reperfused rat hearts. $n = 6, \bar{x} \pm \text{SD}, ***P < 0.01$ vs control (A₂). A₁ = normal.

缺血再灌注心肌 SOD 活力显著低于正常心肌。保护心肌缺血再灌注损伤有效浓度的 PNS 及其单体 R_{b1}、R_{r1} 都减轻 SOD 活力降低的程度, 与钙通道阻滞剂 Ver、黄嘌呤氧化酶抑制剂别嘌呤醇作用一致。

2 心肌 MDA 生成 按照 TBA 比色法^[12]

测定心肌脂质过氧化产物 MDA 含量 (Fig 2)。

在缺血再灌注心肌, MDA 含量较正常心肌高 2 倍。黄嘌呤氧化酶抑制剂别嘌呤醇显著减少 MDA 生成, PNS 12.5 mg/L, R_{b1} 10 mg/L, R_{r1} 10 mg/L 及 Ver $2 \mu\text{mol/L}$ 都有类似作用。

DISCUSSION

我们在整体和离体实验证明, PNS 及其单体 R_{b1}、R_{r1} 对心肌缺血再灌注损伤具有保护作用, 但与缺血时间有关。在缺血早期的心肌有效, 而较长时间缺血引起较严重心肌损伤时作用不显著, 提示与减轻和延缓缺血引起的损伤进程有关。

本文结果表明, 三七皂甙在抗氧自由基这一保护缺血心肌的重要途径有显著作用。PNS 及其单体 R_{b1}、R_{r1} 保护缺血再灌注心肌的 SOD 活力, 增强了内源性氧自由基清除系统的功能; 减少氧自由基作用于膜脂质生成的脂质过氧化产物 MDA, 说明减轻了氧自由基对心肌的损伤。

本文证明, 钙通道阻滞剂 Ver 具有显著的保护 SOD 活力, 减少 MDA 生成的作用。在心肌缺血期, 细胞内游离钙浓度增高, Ca²⁺ 可以激活一种催化黄嘌呤脱氢酶转变为黄嘌呤氧化酶的蛋白水解酶, 加强氧自由基生成的黄嘌呤氧化酶途径; 而氧自由基引起的膜脂质过氧化反应, 可使膜对 Ca²⁺ 的通透性特异性增加^[13], 进一步加剧细胞内钙聚集, 形成恶性循环, 加速细胞内一系列病理改变。Ver 通过减少缺血再灌注心肌的 Ca²⁺ 内流, 抑制黄嘌呤氧化酶形成, 同时降低心肌能量消耗, 保护内源性氧自由基清除剂 SOD 活力, 发挥抗氧自由基作用。三七皂甙已经证实为钙通道阻滞剂, 抗氧自由基可能是其抗钙作用的一个方面。

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中国药理学报 *Acta Pharmacologica Sinica* 1990 Jan; 11 (1) : 29-32

三七总皂甙升高颈动脉前列腺素 I₂ 及降低血小板血栓素 A₂ 的作用¹

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Effects of total saponins of *Panax notoginseng* on increasing PGI₂ in carotid artery and decreasing TXA₂ in blood platelets¹

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ABSTRACT Total saponins of *Panax notoginseng* (PNS) were given orally 100 mg/(kg·d) to rabbit for 8 wk. Aortic atherosclerotic plaque formation was restrained as compared to the control group. Radioimmunoassay was used to investigate the effects of PNS on the contents of prosta-

cyclin in carotid artery and thromboxane A₂ in blood platelets of rat. Oral administration of PNS 25, 50, 100 mg/(kg·d) for 10 d, the caused an increase of prostacyclin in carotid artery and a decrease of thromboxane A₂ in blood platelets as compared to the control group. These results show that the anti-atherosclerotic action of PNS may be a result of the correction of the unbalance between prostacyclin and thromboxane A₂.

KEY WORDS *Panax notoginseng*; ginseng; saponins; atherosclerosis; prostaglandins X; thromboxane A₂; carotid arteries; blood platelets

摘要 三七总皂甙(PNS)100 mg/kg 8 wk 抑制兔实验性动脉粥样硬化(AS)病变形成。用放射免疫法分析 PNS 对大鼠动脉壁 PGI₂ 合成及血小板 TXA₂ 含量的影响。结果分别给 PNS 25, 50, 100 mg/kg 连续

Received 1988 Sep 12 Accepted 1989 Aug 14
¹Project supported by the Science Foundation of the Chinese Academy of Sciences No 3860706