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Protective effects of panaxadiol saponins on cardiac functions in burned rats

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AIM: To study the effects of panaxadiol saponins (PDS) on burn rat heart functions and try to find its mechanisms. **METHODS**: A 35 % skin-full-thickness burn was produced by using napalm in Wistar rats. PDS 30 mg kg^{-1} was injected ip to rats immediately after burn and repeated 2 h before examination. Using the isolated perfused working heart and biochemistry methods, heart rate (HR), cardiac output (CO), coronary flow (CF), left ventricular pressure (LVP), aortic pressure (AP), $\pm dp/dt_{max}$, and content of malondialdehyde (MDA), activity of superoxide dismutase (SOD) in ventricular myocardium homogenate were examined 8 h after burn. **RESULTS**: After burn, HR, CO, CF, LVP, AP, $+dp/dt_{max}$, $-dp/dt_{max}$, and SOD activity decreased from 206 bpm, 92 mL min^{-1} g^{-1}, 26 mL min^{-1} g^{-1}, 7 kPa, 5.9 kPa, 149 kPa s^{-1}, 73 kPa s^{-1}, 2.9 NU/mg protein to 162 bpm, 72 mL min^{-1} g^{-1}, 14 mL min^{-1} g^{-1}, 4 kPa, 2.2 kPa, 77 kPa s^{-1}, 44 kPa s^{-1}, 1.7 NU/mg protein, respectively, and MDA content raised from 0.77 nmol/mg protein to 1.35 nmol/mg protein (P all < 0.05). But in PDS-treated group, above decreased or increased dates restored to 202 bpm, 91 mL min^{-1} g^{-1}, 25 mL min^{-1} g^{-1}, 6 kPa, 4.1 kPa, 112 kPa s^{-1}, 62 kPa s^{-1}, 2.8 NU/mg protein, 0.91 nmol/mg protein, respectively (P all < 0.05 *vs* burn). **CONCLUSION**: PDS exerts definite protective effects on the cardiac functions after burn injury possibly through its enhancement of SOD activity and the reduction of both the levels of free radicals and lipid peroxides (LPO) of the myocardium.

KEY WORDS ginseng; saponins; burn; heart; malondialdehyde; superoxide dismutase

Even in the early stage after serious burn injury, the intrinsic contractility of the myocardium was damaged showing significant decrease of contractile force and cardiac output[1-3], while panaxadiol saponins (PDS) possessed antishock and antiarrhythemia effects. This study was designed to observe the effects of PDS on cardiac functions after burn injury in rats and to probe into the possible mechanisms of the effects.

MATERIALS AND METHODS

Rats Wistar rats ($n=54$) of either sex, weighing $200 \pm s$ 15 g, provided by the Center of Laboratory Animals of our College, were kept in our laboratory for 1 wk prior to the examination. Then they were randomized into the control, burn, and PDS-treated group.

Reagents PDS were extracted from the leaf and stem of *Panax ginseng* C A Meyer (purity >92 %) containing Rb_1, Rb_2, Rb_3, R_c, and R_d, purchased from the Department of Organic Chemistry, Norman Bethune University of Medical Science, analyzed with HPLC and thin layer sweep technic. It was dissolved in normal saline to make a 12.5 g L^{-1} solution.

Superoxide dismutase (SOD, Sigma); xanthin oxydase (Sigma); hypoxanthine (First Reagent Factory of Shanghai), 1, 1, 3, 3-tetraethoxy propane (Fluka); sodium thiobarbital (Xi-an Chemical Industry); sodium dodecyl sulfate (First Reagent Factory of Shanghai); 1-naphthylamide (Beijing Jinxing Chemical

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Industry); hydrochloro-hydroxylamide (Chongqing Dongfang Reagent Factory).

Apparatus LMS-2B polygraphic apparatus (Chengdu Instrument Factory); DU-7H ultraviolet spectrophotometer (Beckman, USA).

Methods The rats were anesthetized with ether and the back and two sides of the trunk were shaved and depilated with 10 % sodium sulfide. Then 35 % skin full thickness burns was inflicted with napalm burning and the depth of burns was confirmed histologically. Examination was started at 8 h after burns.

PDS 30 mg kg^{-1} was injected ip to the rats of the treated group immediately after burn and repeated 2 h before examination. Equal volume of normal saline was given to the rats of other groups instead of PDS. After burn, no fluid injection was allowed to any of the rats. All the rats were kept in separate cages without limitation of water and food intake.

The technic of isolated perfused heart of rats established in our laboratory[4] was used.

Protein[5], malondialdehyde (MDA)[6], and the activity of SOD[7] in ventricular myocardial homogenate were determined.

Statistic analysis Data were treated with ANOVA.

RESULTS

Heart rate (HR), cardiac output (CO), and coronary flow (CF) were decreased in the burn group but were around the control levels in the PDS-treated group. Dry/wet (D/W) rate showed no marked difference among the 3 groups (Tab 1).

Left ventricular pressure (LVP), $+dp/dt_{max}$, and $-dp/dt_{max}$ were decreased in the burn group. In the PDS-treated group, LVP was increased, but was still lower than that of the controls, $\pm dp/dt_{max}$ of the PDS-treated were far higher than that of the burn group; $-dp/dt_{max}$ returned nearly to the level of controls, but $+dp/dt_{max}$ was still lower than the normal level (Tab 1).

Aortic pressure (AP) was decreased after burn. PDS therapy elevated AP which was marked different from that of the burn group, but still lower than that of the controls. The changes of AP were consistent with those of LVP and $+dp/dt_{max}$ (Tab 1).

In the burn group, MDA content in the ventricular myocardial homogenate was increased but SOD activity decreased and both the values were different from those of the control. In the PDS-treated group, there was decrease of MDA content and an increase of SOD activity approaching the normal values (Tab 1).

Tab 1. Effects of PDS (30 mg kg^{-1}, ip) treatment after burn on heart rate (HR), cardiac output (CO), coronary flow (CF), dry/wet rate (D/W), left ventricular pressure (LVP), $\pm dp/dt_{max}$ aortic pressure (AP), malondialdehyde (MDA), and superoxide dismutase (SOD) in ventricular myocardium homogenate of rats. $n=12$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ vs control; $^fP<0.01$ vs burn.

	Control	Burn	Burn+PDS
HR/bpm	206±11	162±40^c	202±17^f
CO/mL min^{-1} g^{-1}	92±9	72±17^c	91±11^f
CF/mL min^{-1} g^{-1}	26±5	14±6^c	25±7^f
D/W	0.17±0.005	0.17±0.007	0.17±0.007
LVP/kPa	7±1	4±1^c	6±1^{bf}
$+dp/dt_{max}$, kPa s^{-1}	149±28	77±25^c	112±19^{bf}
$-dp/dt_{max}$, kPa s^{-1}	73±25	44±10^c	62±10^f
AP/kPa	5.9±1.6	2.2±1.5^c	4.1±1.1^{bf}
MDA ($n=6$), nmol/mg protein	0.77±0.02	1.35±0.10^c	0.91±0.01^f
SOD ($n=6$), NU/mg protein	2.9±0.4	1.7±0.3^c	2.8±0.3^f

DISCUSSION

It was reported in our previous work [1] that the intrinsic myocardial contractility of guineapigs was decreased in the first 24 h after 35 % skin full thickness burns, especially in the 8 h at which the cardiac functions were the lowest. This view agrees to those of other authors[8,9]. In this study we attempt to assess the therapeutic effects of PDS on the cardiac damages resulting from burn injury and to probe into the mechanisms of the therapeutic effects. It was showed that there was little difference of D/W at the end of our experimentation, which excludes the interference of myocardial edema resulting from perfusion on the experimental results.

It was found that MDA content was markedly increased and SOD activity decreased after burn injury, which implies that free radicals producing in burn injury are an important factor to result in myocardial damage and cardiac dysfunction whereas the decrease of SOD activity leads to the disability to scavenge the free redicals and thus aggravate myocardial damages.

It was reported[10] that the direct action of PDS on myocardium is inhibitory. However, we found that PDS ip could evidently improve the functions of an isolated perfused heart of the rats with burn injury. This shown that PDS possesses comprehensive protective effects on the myocardium against burn injury.

How do we explain the contradictory facts that direct PDS effects are inhibitory on myocardium while ip PDS enhances its contractility? It is postulated that PDS might exercise its protective effects on the myocardium during burn injury through its enhancement on SOD activity to scavenge free radicals and its inhibition of the production of LPO. Thus the organization of the cell membrane of the myocardium and other organs is stabilized to alleviate the myocardial damages and improve the cardiac functions after burn injury. Besides, it was found that the restoration of the myocardial contractility after PDS adminstration in burn injury was incomplete. This fact may be explained by partially offsetted by the direct inhibitory effects of PDS on cardiac contractility.

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人参二醇组皂甙对烧伤大鼠心功能的保护作用

R644.05

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目的：研究人参二醇组皂甙(PDS)对烧伤大鼠心功能的影响及其机制. **方法**：测定离体心功能，丙二醛(MDA)含量，超氧化物歧化酶(SOD)活力. **结果**：烧伤后，心率(HR)，心输出量(CO)，左室内压(LVP)及其最大变化率($\pm dp/dt_{max}$)，SOD活力分别由206 bpm，92 mL min^{-1} g^{-1}，7 kPa，149 kPa s^{-1}，73 kPa s^{-1}，2.9 NU/mg 蛋白分别降低至 162，72，4，77，44，1.7，MDA 含量由0.77 nmol/mg 蛋白升至 1.35. PDS 30 mg kg^{-1} ip 使烧伤后降低或升高的上述指标恢复或接近正常. **结论**：PDS 对烧伤心功能保护作用的机制与 MDA 含量降低以及 SOD 活性增高有关.

关键词 人参；皂苷类；烧伤；心脏；丙二醛；超氧化物歧化酶

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3,4-Diaminopyridine facilitates norepinephrine release in chick sympathetic neurons[1]

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AIM: To study the mechanism by which 3,4-diaminopyridine (DAP) facilitates electrically evoked [^{3}H]norepinephrine ([^{3}H]NE) release in sympathetic neurons from chick embryos. **METHODS**: The neurons were incubated with [^{3}H]NE or Fura-2. [^{3}H]NE release or [Ca^{2+}]$_i$ was determined. **RESULTS**: The electrically evoked [^{3}H]NE release and the elevation of [Ca^{2+}]$_i$ were inhibited completely by sodium channel blocker tetrodotoxin (TTX), strongly by the antagonist of N-type calcium channel ω-conotoxin GVIA (CTX), and slightly by the antagonist of L-type calcium channel (−)isradipine, but enhanced by the agonist of L-type calcium channel Bay k 8644. In the presence of DAP, the electrically evoked [^{3}H]NE release and [Ca^{2+}]$_i$ were facilitated, the inhibition of [^{3}H]NE release and [Ca^{2+}]$_i$ by CTX was attenuated, but that by (−)isradipine was enhanced, and Bay k 8644 was no longer effective. **CONCLUSION**: In the cultured chick sympathetic neurons DAP facilitates electrically evoked [^{3}H]NE release mediated by enhancement of influx of external Ca^{2+} through the L-type Ca^{2+} channel.

KEY WORDS sympathetic neuron; norepinephrine; 3,4-diaminopyridine; calcium channel; ω-conotoxins; tetrodotoxin

The discovery that calcium is necessary for the release of neurotransmitter led to the calcium hypothesis of neurotransmitter release[1] in which release is initiated after an action potential by an increase in intracellular calcium concentration near the release sites.

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