BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 May; 17 (3): 245 - 248

# Diacetylamethystoidin A protects isolated working rat heart against myocardial reperfusion injury

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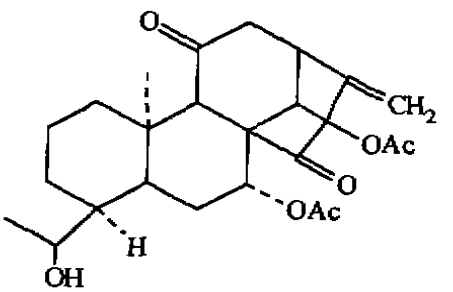
**KEY WORDS** diacetylamethystoidin A; myocardial reperfusion injury; lipid peroxidation

**AIM**: To observe the effect of diacetylamethystoidin A (6α, 13β-diacetyl-11, 15-dioxo-19-hydroxyl-kaurene, DAA-A) on myocardial ischemia reperfusion injury. **METHODS**: Working heart mode was induced in isolated rat heart subjected to a 40-min ceasing perfusion followed by a 25-min reperfusion. **RESULTS**: DAA-A 0.13, 0.25, 0.50 mmol·L$^{-1}$ reduced the incidence of ventricular fibrillation (1, 1, 0 vs 4 in Vehicle and 5 in Control, respectively), increased the coronary flow (mL·min$^{-1}$, 1.8±0.9, 1.9±0.8, 1.7±0.6 *vs* 1.0±0.3 in Vehicle and 0.9±0.5 in Control as reperfusion time 5 min, respectively), improved the contractile function of heart, decreased the release of lactic dehydrogenase (LDH) and malondialdehyde (MDA). DAA-A 0.25 mmol·L$^{-1}$ reduced the injury of myocardial cell ultrastructure. **CONCLUSION**: DAA-A has cardioprotective effect through diminishing cellular lipid peroxidation induced by oxygen free radicals.

Diacetylamethystoidin A (6α, 13β-diacetyl-11, 15-dioxo-19-hydroxyl-kaurene, DAA-A), a new amethystoidin derivative, has antibacterial activities *in vitro* and *in vivo*[1,2], antitumor activity[3,4], inhibitory effect on CaM-dependent phosphodiesterase[5]. We found that DAA-A acted against the arrhythmias induced by isoprenaline, ouabain and aconitine[6]. But there is no information about the effect of DAA-A on ischemia reperfusion injury. In the present study, the effect of DAA-A on myocardial reperfusion injury in isolated working rat hearts were examined.




**Diacetylamethystoidin A (DAA-A)**

Received 1993-07-09 Accepted 1995-09-18

## MATERIALS AND METHODS

**Drugs** DAA-A, extracted by Phytochemical Laboratory of Nanjing Institute of Materia Medica, $M_r$ = 432.5, purity >99 %, 1 g DAA-A was dissolved in 1 L vehicle (5 % Tween-80 and 4 % PVP). Thiobarbituric acid: Fluka AG Buches, Switzerland.

**Working-heart preparation** Wistar rats ($n$ = 50) weighing 240 ± $s$ 28 g were anesthetized with sodium pentobarbital (45 mg·kg$^{-1}$ ip). The hearts were first perfused by the Langendorff technique[7] for 15 min under a pressure of 5.88 kPa, switched to the working mode and equilibrated for 10 min, ceased perfusion 40 min, reperfused by Langendorff technique for 15 min, working heart mode for 10 min. In the working heart mode, the perfusate entered the cannulated left atrium at a constant pressure 1.47 kPa and passed into the left ventricle, from which it was spontaneously ejected through the aortic cannula against an after-load pressure of 4.41 kPa. A catheter was inserted into the ventricle to measure left ventricular systolic pressure (LVSP) and ± $dp/dt$. The cardiac parameters: LVSP; ± $dp/dt_{max}$; centuple amplitude (CA); II-lead ECG were recorded with a Four Track recorder (Type RM-6200). Modified Krebs-Henseleit's solution (NaCl: 105.7; KCl: 5.36; $CaCl_2$: 1.28; $MgSO_4 \cdot 7H_2O$: 1.18; $NaHCO_3$: 24.88; $KH_2PO_4$: 1.18; glucose: 11.00 mmol·L$^{-1}$) equilibrated with a gas mixture of 95 % $O_2$ and 5 % $CO_2$ was used as the perfused medium. When the effect of the drug or its vehicle was examined, the hearts were perfused with perfusate containing the drug or vehicle during 15 min before ceasing perfusion and 25 min after reperfusion. The concentration of DAA-A used were 0.13, 0.25, 0.50 mmol·L$^{-1}$, the vehicle's was 10 mL·L$^{-1}$.

**Biochemical assays** The activity of the cytolic enzyme LDH in the coronary effluent was measured by a fluorescent method[8]. The content of malondialdehyde (MDA) in the effluent was examined by the thiobarbituric acid fluorophotometry at 532 nm[9].

**Microscopical examination** At the end of reperfusion, thin slices (0.5 μm) of the left ventricle[10] were cut from at least 3 representative blocks and examined under a Hitachi H-600 transmission electron microscope.

**Statistic analysis** The comparison was made using $t$-test.

## RESULTS

**Arrhythmias** When control hearts were reperfused, ventricular tachycardia (VT) or fibrillation (VF) were consistently induced. Treatment with DAA-A shortened the duration of arrhythmias, reduced the incidence of VF. DAA-A 0.5 mmol·L$^{-1}$ inhibited completely the appearance of VF. However, the vehicle had no effect (Tab 1).

**Tab 1. Effect of diacetylamethystoidin A (DAA-A) on arrhythmia induced by reperfusion in isolated rat hearts.** $n = 10$, $\bar{x} \pm s$. $^{a}P > 0.05$, $^{c}P < 0.01$ *vs* **control.**

| | VF in | Duration of arrhythmia/s |
|---|---|---|
| Control | 5 | 423 ± 59 |
| Vehicle | 4[a] | 409 ± 38[a] |
| DAA-A 0.13 mmol·L$^{-1}$ | 1[c] | 175 ± 30[c] |
| 0.25 mmol·L$^{-1}$ | 1[c] | 147 ± 21[c] |
| 0.50 mmol·L$^{-1}$ | 0[c] | 138 ± 39[c] |

**Cardiac function** In control hearts, induction of ischemia-reperfusion resulted in a rapid decline in coronary flow (CF), LVSP, $\pm dp/dt_{max}$, and CA. Improvement of these parameters were observed in the hearts treated with DAA-A, but not with its vehicle (Tab 2).

**LDH and MDA in coronary effluent** Leakage of LDH (an indicative of the severity of reperfusion injury to the myocardium) and MDA (the end product of lipid peroxidation) into the coronary flow were examined. Large increase in LDH and MDA were observed in control and vehicle hearts. DDA-A blunted the increase in LDH and MDA (Fig 1).

**Myocardial ultrastructure** In control hearts, prominent myofibrillae bands contracted; mitochondria displayed swelling and disruption, there are large amorphous densities in their matrix; the nucleus was shrunken, many vacuoles are present. DAA-A 0.25 mmol·L$^{-1}$ protected the myocardium. The sarcoplasmic reticulum was intact, the myofibrillae were unalterable; some mitochondria showed a slight clearing of matrix, but most mitochondria were dense in the sarcoplasma (Fig 2).

**Tab 2. Effect of DAA-A on cardiac function in isolated working rat hearts.** $n = 10$, $\bar{x} \pm s$.
$^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* **control.**
$^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* **pre-ischemia.**

| | DAA-A mmol·L$^{-1}$ | Reperfusion time/min: Before ischemia | 5 | 10 |
|---|---|---|---|---|
| LVSP, kPa | Control | 5.4 ± 1.7 | 2.1 ± 0.7[f] | 1.9 ± 0.6[f] |
| | Vehicle | 4.3 ± 1.3[a] | 2.0 ± 0.4[af] | 2.0 ± 0.3[af] |
| | 0.13 | 5.1 ± 1.8[a] | 3.4 ± 0.8[be] | 3.0 ± 1.1[bf] |
| | 0.25 | 5.2 ± 0.8[a] | 3.8 ± 1.2[be] | 3.6 ± 1.2[be] |
| | 0.50 | 4.8 ± 1.6[a] | 2.8 ± 1.3[be] | 2.7 ± 1.5[bf] |
| $+dp/dt_{max}$, kPa·s$^{-1}$ | Control | 69 ± 29 | 22 ± 10[f] | 23 ± 8[f] |
| | Vehicle | 66 ± 27[a] | 23 ± 6[af] | 25 ± 7[af] |
| | 0.13 | 76 ± 18[a] | 35 ± 15[be] | 27 ± 17[be] |
| | 0.25 | 79 ± 33[a] | 53 ± 35[ce] | 44 ± 27[ce] |
| | 0.50 | 61 ± 29[a] | 41 ± 25[be] | 35 ± 23[be] |
| $-dp/dt_{max}$, kPa·s$^{-1}$ | Control | 47 ± 26 | 25 ± 8[f] | 24 ± 7[f] |
| | Vehicle | 44 ± 12[a] | 19 ± 3[af] | 19 ± 6[af] |
| | 0.13 | 52 ± 18[a] | 27 ± 5[af] | 24 ± 9[af] |
| | 0.25 | 51 ± 20[a] | 39 ± 21[be] | 36 ± 8[be] |
| | 0.50 | 45 ± 20[a] | 30 ± 18[be] | 28 ± 17[bf] |
| CA, mm | Control | 20 ± 11 | 3.4 ± 2.0[f] | 3.1 ± 2.0[f] |
| | Vehicle | 15 ± 7[a] | 3.4 ± 2.0[af] | 2.9 ± 2.0[af] |
| | 0.13 | 19 ± 7[a] | 13 ± 5[ce] | 12 ± 7[cd] |
| | 0.25 | 19 ± 8[a] | 12 ± 8[cd] | 10 ± 6[ce] |
| | 0.50 | 19 ± 10[a] | 10 ± 6[cd] | 9 ± 5[cd] |
| CF, mL·min$^{-1}$ | Control | 2.7 ± 0.7 | 0.9 ± 0.5[f] | 0.8 ± 0.5[f] |
| | Vehicle | 2.9 ± 0.6[a] | 1.0 ± 0.3[af] | 0.8 ± 0.3[af] |
| | 0.13 | 2.8 ± 1.1[a] | 1.8 ± 0.9[be] | 1.7 ± 0.7[be] |
| | 0.25 | 2.8 ± 0.8[a] | 1.9 ± 0.8[ce] | 2.1 ± 0.9[cd] |
| | 0.50 | 2.8 ± 1.2[a] | 1.7 ± 0.6[be] | 1.6 ± 0.6[be] |

## DISCUSSION

The attenuation of myocardial reperfusion injury as indication by the reduction of LDH release, better preservation of myocardium ultrastructure reinjury suggest that DAA-A has some cardioprotective effect. Further, it reduced the content of MDA, indicating its effectiveness in

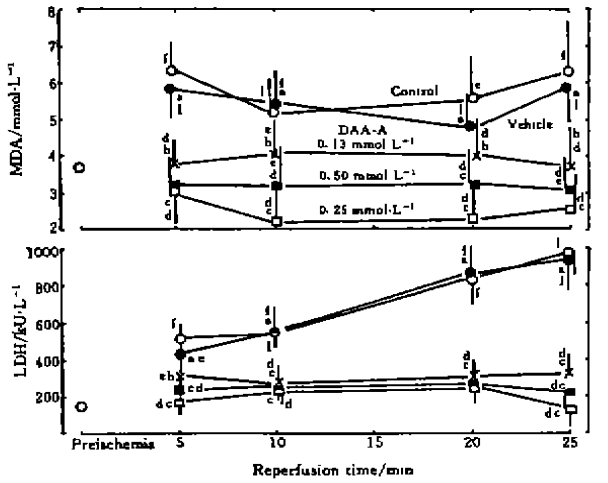



**Fig 1. Effect of DAA-A on LDH and MDA release in isolated rat hearts. $n = 10$.**
**$^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control.**
**$^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* before ischemia.**

interfering with free radicals interaction.

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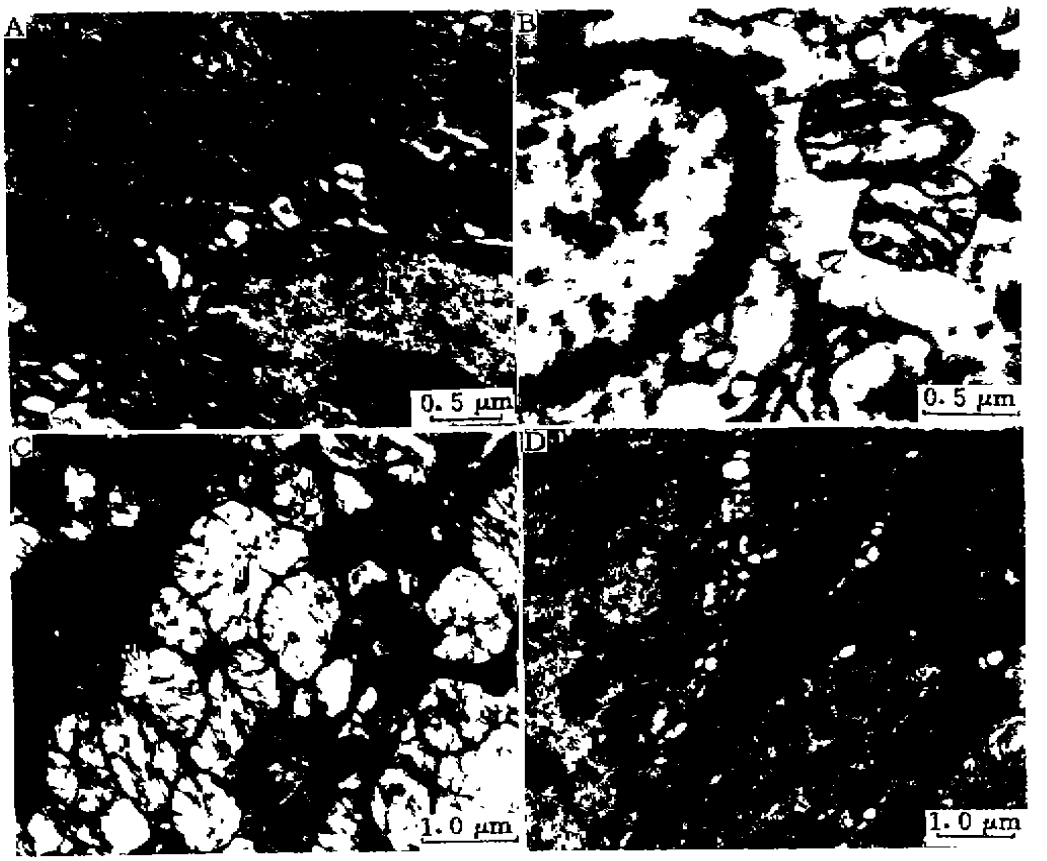


**Fig 2. Effect of DAA-A on myocardial reperfusion injury. A) Normal myocardial ultrastructure. B, C) Control groups: heart exposed to ischemia-reperfusion. D) DAA-A 0.25 mmol·L$^{-1}$ on myocardium.**

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## 双乙酰香茶菜甲素抗离体大鼠工作心脏缺血再灌注损伤的作用

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R963

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关键词 双乙酰香茶菜甲素；心肌再灌注损伤；脂质过氧化

目的：观察双乙酰香茶菜甲素(DAA-A)对心肌缺血再灌注损伤的作用. 方法：离体大鼠心脏停灌 40 min，再灌 25 min，造成心肌缺血再灌注损伤模型. 结果：DAA-A 0.13，0.25，0.50 mmol·L$^{-1}$对再灌注所致心功能低下有心脏保护作用，降低室颤发生率及乳酸脱氢酶(LDH)，丙二醛(MDA)的生成量. DAA-A 0.25 mmol·L$^{-1}$改善心肌超微结构. 结论：DAA-A 抗心肌缺血再灌注损伤的作用与其抗脂质过氧化损伤有关.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 May; 17 (3): 248 - 251

# Effects of daurisoline on intracellular $Ca^{2+}$ activity in myocardium[1]

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**KEY WORDS** microelectrodes; calcium; electrophysiology; myocardium; daurisoline

**AIM**: To explain the effect of daurisoline (DS) on delayed afterdepolarization (DAD). **METHODS**: $Ca^{2+}$-sensitive microelectrode technic was used to record intracellular $Ca^{2+}$ activity ($\alpha^{i}_{Ca}$) and triggered activity (TA) arising from DAD in myocardium. **RESULTS**: Strophantin G 3 μmol·L$^{-1}$ yielded an increase in resting myocardial $\alpha^{i}_{Ca}$ by 0.19 ± 0.11 μmol·L$^{-1}$ and transient elevations of $\alpha^{i}_{Ca}$ by 1.48 ± 0.55 and 4.96 ± 1.81 μmol·L$^{-1}$, respectively during the development of DAD and TA. By pretreatment with DS or verapamil, strophantin G-caused elevations of the $\alpha^{i}_{Ca}$ in resting and provoked myocardia were eliminated and TA disappeared. DS 50 μmol·L$^{-1}$ reduced $Na^{+}$-free medium-induced elevation of dog Purkinje fibrous $\alpha^{i}_{Ca}$ and abolished caffeine-induced increase of dog myocardial $\alpha^{i}_{Ca}$. **CONCLUSIONS**: DS inhibited DAD and TA by preventing an increase of $\alpha^{i}_{Ca}$ via transmembrane $Ca^{2+}$ entry and $Ca^{2+}$ release from the reticulum.

Although a substantial increase of cytoplasmic $Ca^{2+}$ is a prerequisite for delayed afterdepolarizations (DAD) that has been implicated as a cellular mechanism for arrhythmias due to affecting normal impulse conduction and engendering triggered activity (TA)[1], little has been reported in literature on simultaneous measurements of intracellular $Ca^{2+}$ activity ($\alpha^{i}_{Ca}$) and DAD/TA in a same myocardium[2]. A neutral ligand $ETH_{1001}$ $Ca^{2+}$-sensitive microelectrode (Ca-ISE), which developed to have a fine tip and show stable property, ample sensitivity and transient response to free $Ca^{2+}$ in a submicromolar range[3,4], allowed to

[1] Project supported by the National Natural Science Foundation of China, № 39170842.
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Received 1994-07-13 Accepted 1995-06-12