BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Nov; 17 (6): 500 - 502

Effect of procainamide on pulmonary thromboembolism and platelet malondialdehyde in mice

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KEY WORDS procainamide; thrombosis; malondialdehyde; platelet aggregation

AIM: To study the effect of procainamide (PA) on pulmonary thromboembolism, platelet malondialdehyde (MDA) level and platelet aggregation induced by collagen and adrenaline. **METHODS**: Pulmonary thromboembolism, 2-thiobarbituric acid fluorescence micro-determination, conventional microscopic counting, and platelet aggregation test were used. **RESULTS**: PA (10 - 20 mg $\cdot kg^{-1}$) and mannitol (200 mg $\cdot kg^{-1}$) reduced thrombosis by 30 % - 75 % and 75 %, respectively. Thrombocytopenia followed thrombosis increased after the pretreatment of PA and mannitol. MDA decreased by both of them *in vivo*. *In vitro*, PA inhibited platelet aggregation and MDA production induced concentration-dependently by collagen and adrenaline. **CONCLUSION**: Inhibition of PA on pulmonary thromboembolism is involved in the decrease of platelet aggregation and MDA production.

Procainamide (PA) inhibited rabbit platelet aggregation induced by ADP[1], arachidonic acid, thrombin[2], and clonidine[3] *in vitro* or *in vivo*. It inhibited platelet adhesion in rats[4]. Infusion of collagen and adrenaline caused thrombosis of mice[5, 6]. Malondialdehyde (MDA), a lipid peroxidation product, increased in platelets of thrombotic challenge mice[5]. This study was to explore the effect of PA on pulmonary thromboembolism induced by collagen and adrenaline in mice and its possible mechanism.

MATERIALS AND METHODS

Reagent and instrument PA was from Beijing Pharmaceutical Factory. Adrenaline was from Guangming Pharmaceutical Factory. Collagen, 2-thiobarbituric acid (TBA), diamisidine, and 1, 1, 3, 3-tetraethoxypropane (TEP) were purchased from Sigma. Aggregometer was made by Beijing Biopharmaceutical Factory (Model BS 631). The fluorescence was measured using RF-5000 Spectrofluorometer (Shimadzu Japan).

***In vivo* experiment**

Mouse pulmonary thromboembolism Thrombosis was induced by injecting 100 μL of a mixture containing collagen 10 μg and adrenaline 5 μg into the tail vein of ♀ white mice (30 - 35 g, $n = 54$) of Swiss strain[6]. This resulted in death/paralysis of hind limbs in all the mice. The drugs were injected *ip* 1 h prior to the thrombotic challenge.

Platelet preparation Under ether anesthesia jugular vena blood was collected into sodium citrate (129 mmol $\cdot L^{-1}$, 9:1 vol/vol). Platelet-rich plasma (PRP), obtained by centrifuging at 150 × *g* for 15 min, was then centrifugated at 150 × *g* for 20 min. Platelet pellet was suspended in calcium-free Tyrode's buffer (pH 7.4) in which the number of platelets was adjusted to $5 \times 10^{10} \cdot L^{-1}$.

Platelet MDA TBA fluorescence micro-determination was adopted for measurement of MDA[7], modified for platelets. Standard curve was drawn with TEP. The platelet suspension was lysed by repeated freezing and thawing for MDA estimation. Fluorescence was measured at 25 ℃ (λ_{ex}) 532 nm, (λ_{em}) 553 nm.

Platelet count Platelets were counted under conventional microscope. Blood was collected by cutting the tip of tail 3 - 5 min after thrombotic challenge.

***In vitro* experiment**

Platelet aggregation A model BS 631 aggregometer was used for platelet aggregation measurement. Aliquots (0.5 mL) of preparated platelet suspension, which had been incubated with PA at 37.0 ℃ for 15 min, were placed in siliconized glass tubes and warmed to 37.0 ℃ for 5 min without stirring. $CaCl_2$ 1 mmol $\cdot L^{-1}$ (final concentration) was added 2 min prior to the addition of collagen and adrenaline. The curve of platelet aggregation was recorded[1].

Platelet MDA MDA was measured as described *in vivo* experiment 5 min after platelet aggregation instead of lysing platelets.

Statistics *Chi*-square test or *t* test.

RESULTS

PA reduced the collagen- and adrenaline-

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Received 1995-03-28 Accepted 1996-04-01

induced deaths, which was dose-related. At 10 and 20 mg·kg^{-1}, PA offered protection against thrombosis by 30 % and 75 % respectively. Mannitol (200 mg·kg^{-1}) exhibited protection by 75 % (Tab 1).

Tab 1. Effects of procainamide (PA) and mannitol on pulmonary thromboembolism.
$^{a}P>0.05$, $^{c}P<0.01$ *vs* control.

Pretreatment	Mice	Died	Survival/%
Control	10	10	0
PA 10 mg·kg^{-1}	20	14	30^{a}
PA 20 mg·kg^{-1}	12	3	75^{c}
Mannitol 200 mg·kg^{-1}	12	3	75^{c}

Platelet count in normal mouse blood was (220 ± 26) · 10^{9} · L^{-1}. Thrombosis was followed by 25 % thrombocytopenia in the control group. Compared with control group, platelet count increased by 12 % and 18 % in the presence of PA (20 mg·kg^{-1}) and mannitol (200 mg·kg^{-1}), respectively (Tab 2).

Tab 2. Effects of PA 20 mg·kg^{-1} or *mannitol* 200 mg·kg^{-1} on circulatory platelet counts and platelet malondialdehyde (MDA) level after pulmonary thrombotic challenge (TC). *n* = Number of mice.
$\bar{x} \pm s$. $^{b}P<0.05$ *vs* control.

Pretreatment	TC	Platelet counts (n) (·10^{10}·L^{-1})	MDA (n) (nmol·L^{-1}/2·10^{7} cell)
Saline	−	22.0 ± 2.6 (10)	24.4 ± 6.2 (5)
	+	16.4 ± 2.7 (10)	45.9 ± 13.7 (6)
Procainamide	+	18.5 ± 2.0^{b} (12)	33.0 ± 7.3^{b} (8)
Mannitol	+	19.4 ± 1.9^{b} (10)	30.2 ± 5.1^{b} (7)

Level of MDA in normal group was 24 ± 6 nmol·L^{-1}/2·10^{7} cell (Tab 2). It increased by 88 % in the control groups after thrombosis. However, levels of MDA, compared with control group, were decreased by 28 % and 35 % in the presence of PA (20 mg·kg^{-1}) and mannitol (200 mg·kg^{-1}), respectively (Tab 2).

PA diminished the platelet aggregation induced by collagen and adrenaline *in vitro*, except for 13.8 μmol·L^{-1} concentration (Fig 1).

Levels of MDA produced by platelet aggregation *in vitro* were decreased by PA at doses of 13.8, 55.2, and 220.8 μmol·L^{-1}. A linear correlation between PA and MDA was shown ($r = -0.9908$, $P < 0.01$) (Tab 3). But lower concentration (13.8 μmol·L^{-1}) had no significant effect.

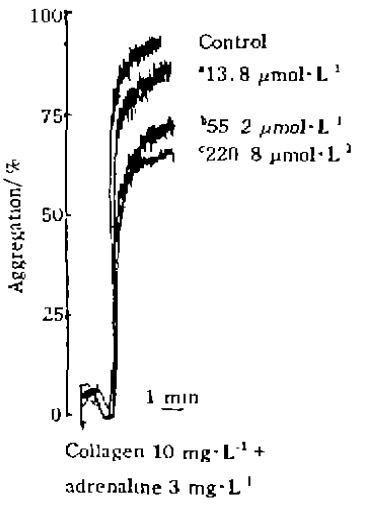

Fig 1. Effect of PA on platelet aggregation induced by collagen and adrenaline *in vitro*. $n = 5$, $\bar{x} \pm s$.
$^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

Tab 3. Effect of PA on MDA level after platelet aggregation induced by collagen and adrenaline *in vitro*. $n = 5$, $\bar{x} \pm s$.
$^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

PA (μmol·L^{-1})	MDA (nmol·L^{-1}/2·10^{7} cell)
0	145.1 ± 16.8
13.8	134.1 ± 28.1
55.2	112.5 ± 18.1^{a}
220.8	82.9 ± 14.5^{c}

DISCUSSION

In this study, we have demonstrated the protection of PA against pulmonary thromboembolism induced by collagen and adrenaline in mouse. PA (20 mg·kg^{-1}) exhibited a similar effect against thrombisis to mannitol (200 mg·kg^{-1}). The formation of thrombosis is due to platelet aggregation in the lung microcirculation. Many platelet modifying agents, such as cyclooxygenase inhibitors, calcium antagonists and free radical scavenger have antithrombotic effect[18]. The

further mechanism that PA inhibited thrombosis and platelet aggregation induced by collagen and adrenaline has not been well established, but it should be related to inhibition of PA on MDA, TXA_2 product[2] in platelet.

In conclusion, inhibition of PA on pulmonary thromboembolism is involved in the decrease of platelet aggregation and production of MDA.

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普鲁卡因胺对小鼠肺栓塞和血小板 MDA 生成的影响

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关键词 普鲁卡因胺；血栓形成；丙二醛；血小板聚集

目的：探讨普鲁卡因胺（PA）对肺栓塞，血小板丙二醛（MDA）及血小板聚集的影响 **方法**：建立小鼠肺栓塞模型，用 TBA 荧光微量测定法检测 MDA，计数血小板并进行血小板聚集测定 **结果**：PA（10 - 20 mg·kg^{-1}）和甘露醇（200 mg·kg^{-1}）对小鼠肺栓塞的保护作用分别为 30 % - 75 % 和 75 % 肺栓塞模型建立后循环血小板数减少可因 PA 和甘露醇预先给药而部分回升. 两者均能抑制肺栓塞后血小板 MDA 的升高 体外实验发现，PA 抑制胶原 + 肾上腺素诱导的血小板聚集和 MDA 生成，呈良好的量效关系 **结论**：PA 抑制小鼠肺栓塞与其抑制血小板聚集，降低血小板 MDA 有关