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Inhibitiory effects of procainamide on rabbit platelet aggregation and thromboxane B_2 production *in vitro*

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KEY WORDS procainamide; platelet aggregation; thromboxane B_2; thrombin

AIM: To study the influences of procainamide (PA) on thrombin-induced rabbit platelet aggregation and thromboxane B_2 (TXB_2) production *in vitro*. **METHODS**: Turbidimetry and radioimmunoassay were used. **RESULTS**: PA 8.5, 34, 136, and 544 $\mu mol \cdot L^{-1}$ inhibited thrombin-induced platelet aggregation and TXB_2 production, and the inhibitory rates were 45 % ± 37 %, 48 % ± 32 %, 88 % ± 23 %, 92 % ± 15 % and 53 % ± 24 %, 65 % ± 26 %, 90 % ± 6 %, 95 % ± 6 %, respectively. There was positive correlation between PA concentration and efficiency of inhibition of platelet aggregation and TXB_2 production, and also between the inhibition % of platelet aggregation and that of production of TXB_2. The three linear equations and main parameters were $\hat{Y} = 0.2075X - 4.9157$, $r = 0.9985$; $\hat{Y} = 0.9546X - 34.6724$, $r = 0.9921$; $\hat{Y} = 0.8202X + 19.7062$, $r = 0.9921$. **CONCLUSION**: PA inhibited thrombin-induced platelet aggregation and TXB_2 production in rabbits.

Procainamide (PA) inhibited the action of many platelet aggregation-inducers, such as adenosin diphosphate (ADP), arachidonic acid (AA), $CaCl_2$, and α_2-adrenergic receptor agonist — clonidine. PA inhibited thromboxane B_2 (TXB_2) production in AA-induced platelet aggregation[1-3]. PA also reduced platelet adhesion[4], and inhibited pulmonary thromboembolism and malondialdehyde (MDA) production[5]. PA inhibited thrombin-induced platelet aggregation[2]. Therefore, this study was to explore the effect of PA on thrombin-induced platelet aggregation and TXB_2 production.

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MATERIALS AND METHODS

Reagents PA was bought from Beijing Pharmaceutical Factory. Thrombin was obtained from Shanghai Huashan Hospital. Ethyl acetate was purchased from Shuanglin Chemical Industrial Reagent Factory, Hangzhou, Zhejiang. Scintillation liquid was composed of 2,5-diphenyloxazole (PPO) 4 g, 1,4-bis-(5-pheny-2-oxazolyl) benzene (POPOP) 20 mg and naphthalene 60 g dissolved in 1000 mL of toluene, and to which absolute ethanol 570 mL was added after standing over night. TXB_2 radioimmunological kit was bought from General Hospital of the Chinese People's Liberation Army (PLA). Aggregometer, Model BS631, was purchased from Beijing Biopharmaceutical Factory. Liquid Scintillation Counter, LS9800, was bought from Beckman.

Platelet aggregation experiment[2,3] New Zealand rabbits of either sex ($n = 8$, weighing $2.2 \pm s$ 0.4 kg) were bred by the Experimental Animal Center of First Military Medical University, Guangzhou. Platelet rich plasma (PRP) and platelet pellets were prepared. The pellets were resuspended in Ca^{2+}-free Tyrode's solution, in which the number of platelets was usually adjusted to $5.0 \times 10^{11} \cdot L^{-1}$.

Turbidimetric tubes, 48 in number (each tube contained platelet suspension 0.5 mL), were divided into 6 groups. Group A without other substance in addition to platelet suspension. Group B with the same volume of normal saline (NS) as in groups C, D, E, and F. In groups C – F, the concentrations of PA were 8.5, 34, 136, and 544 $\mu mol \cdot L^{-1}$, respectively. Except in group A, all tubes were placed in the aggregometer and incubated for 2 min. The thrombin was added as inducer in each tube. The curves of absorbance by thrombin (final concentration 100 $IU \cdot L^{-1}$)-induced platelet aggregation were recorded.

TXB_2 extraction and determination[6,7] Group A platelet suspension and groups B – F thrombin-induced platelet suspensions were immediately placed in ice into which HCl (final concentration 0.09 $mmol \cdot L^{-1}$) was added to terminate platelet aggregation and TXB_2 production. Redistilled ethyl acetate was used to extract TXB_2 which was then stored under – 20 ℃ before measurement. Cpm was recorded with LS9800 Liquid Scintillation Counter. TXB_2 values (μg/tube) of the samples were calculated according to a standard curve drawn with standard TXB_2. TXB_2 values ($\mu g \cdot L^{-1}$) of groups B – F minus that of group A were the production TXB_2 ($\mu g \cdot L^{-1}$) in

thrombin-induced platelet aggregation. The inhibitory percentage of platelet aggregation and TXB_2 production was calculated by the following formulas:

Platelet aggregation inhibition (%) = [Curve of absorbance (mm) of NS group – that of drug group]/that of NS group.

TXB_2 inhibition (%) = (TXB_2 values of NS group – that of drug group)/that of NS group.

Data analysis The data were expressed as $\bar{x} \pm s$ and analyzed with t test. The correlations between PA concentrations and inhibitory efficacies of platelet aggregation and TXB_2 production were calculated according to Scott's method[8].

RESULTS

PA 8.5 – 544 $\mu mol \cdot L^{-1}$ inhibited the thrombin-induced platelet aggregation and TXB_2 production (Tab 1).

Tab 1. Influences of procainamide (PA) on thrombin-induced rabbit platelet aggregation and thromboxane B_2 (TXB_2) production *in vitro*. $n = 8$ rabbits, $\bar{x} \pm s$. $^cP < 0.01$ *vs* normal saline (NS).

Drug/ $\mu mol \cdot L^{-1}$	Platelet aggregation		Production of TXB_2	
	Curve of absorbance/ mm	Inhibition/ %	Amount/ $\mu g \cdot L^{-1}$	Inhibition/ %
NS	61 ± 13		29 ± 14	
PA				
8.5	30 ± 15^c	45 ± 37	12 ± 6^c	53 ± 24
34.0	29 ± 15^c	48 ± 32	10 ± 9^c	65 ± 26
136.0	7 ± 13^c	88 ± 23	2.6 ± 1.5^c	90 ± 6
544.0	5 ± 9^c	92 ± 15	1.1 ± 0.8^c	95 ± 6

There were positive correlations between PA concentrations and inhibitory efficiencies of platelet aggregation and TXB_2 production ($r = 0.9985$, $P < 0.01$; $r = 0.9921$, $P < 0.01$) and also between the inhibition percentages of platelet aggregation and that of TXB_2 production ($r = 0.9921$, $P < 0.01$) (Fig 1 – 3).

DISCUSSION

We have previously reported inhibition of thrombin-induced platelet aggregation by PA[2], but have not reported the inhibition of TXB_3 production by PA and the correlation between them, TXA_2 is a highly unstable compound which half life ($T_{1/2}$) is only 30 s. However, its metabolite is a highly stable substance, TXB_2. Therefore, the measured TXB_2 value represents

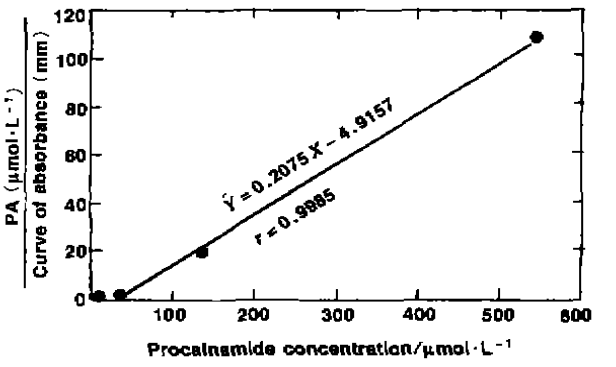

Fig 1. Correlation between PA concentration and platelet aggregation (Scott's method). $n = 8$ rabbits.

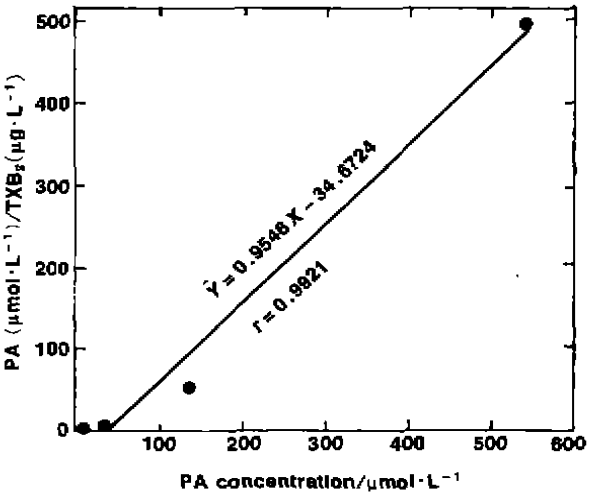

Fig 2. Correlation between PA concentration and TXB_2 production (Scott's method). $n = 8$ rabbits.

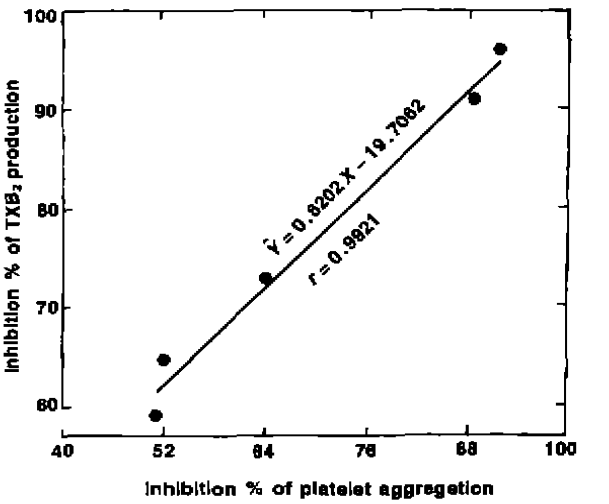

Fig 3. Correlation between inhibitory percentage of platelet aggregation and inhibitory percentage of TXB_2 production.

TXA_2 value. The dense tubular system in platelet can release calcium ion when thrombin, collagen, ADP, and other induce platelet aggregation and cause releasing reaction of platelets. Calcium ion activates phospholipase A_2 which promotes the AA release from phospholipid of platelet membrane, potentiating the formation of TXA_2[9].

In this paper, the inhibition of thrombin-induced platelet aggregation and TXB_2 production by PA resulted probably from blockade of PA on thrombin-receptors on platelet membrane, blocking the releasing Ca^{2+} in the dense tubular system. At the same time, PA also *inhibited thrombin-induced* Ca^{2+} *influx*[10,11]. Thus, free calcium was reduced in platelet, inhibiting phospholipase A_2 activation. The released AA was reduced from phospholipid of platelet membrane, reducing TXA_2 production.

Inhibition by PA was dose-dependent, and according to Scott's method, there were positive correlations between PA concentrations and inhibitory efficacies of platelet aggregation, and that of TXB_2 production. Also there was positive correlation between inhibition of platelet aggregation and that of TXB_2 production.

REFERENCES

1 Shan CW, Hu ZJ, Liang QZ, Liu JF. Inhibitory action of procainamide on blood platelet aggregation of rabbits and guinea pigs. Acta Pharmacol Sin 1986; 7: 63 – 4.

2 Shan CW, Liang QZ. Effects of procainamide on blood platelet aggregation in rabbits. Acta Pharmacol Sin 1988; 9: 338 – 41.

3 Shan CW, Yang JW, Yang SQ. Blockage of clonidine-induced platelet aggregation in rabbits by procainamide. Acta Pharmacol Sin 1993; 14: 127 – 9.

4 Yang YS, Zhou YX, Ma FB, Shan CW. *Effect of procainamide on platelet adhesion in rats.* Acta Pharmacol Sin 1994; 15: 339 – 40.

5 Pang JX, Sun LS, Wang XY, Yang SQ, Shan CW. Effect of procainamide on pulmonary thromboembolism and platelet malondialdehyde in mice. Acta Pharmacol Sin 1996; 17: 500 – 2.

6 Shan CW, Yang SQ, He HD, Shao SL, Zhang PW. Influences of 3,4,5-trihydroxystibene-3-β-mono-*D*-glucoside on rabbits' platelet aggregation and thromboxane B_2 production *in vitro*. Acta Pharmacol Sin 1990; 11: 527 – 30.

7 Granström E, Kindahl H, Samuelesson B. Radioimmunoassay for thromboxane B_2. Anal Lett 1976; 9: 611 – 27.

8 Sun RY. Quantitative pharmacology. 1st ed. Beijing: People's Health Press; 1987. p 404 – 5.

9 Ruan CG. Platelet physiological function. In: Ruan CG, editor. Blood platelet — foundation and clinic. 1st ed. Shanghai: Shanghai Scientific Technical Publishers; 1987. p 38 – 59.

10 Pang JX, Shan CW. Effect of procainamide on cytosolic free Ca^{2+} induced by thrombin in single human platelet. Acta Pharmacol Sin 1996; 17: 87.

11 Pang JX, Shan CW. Procainamide inhibits A23187-induced human platelet aggregation and increase in cytosolic free-Ca^{2+} in the cells. J Med Coll PLA 1996; 11: 241 – 3.

164-166

普鲁卡因胺在体外对兔血小板聚集和血栓素 B_2 生成的抑制作用

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关键词 普鲁卡因胺; 血小板聚集; 血栓素 B_2; 凝血酶

目的: 研究普鲁卡因胺(PA)对凝血酶诱导血小板聚集和血栓素 B_2(TXB_2)产生的影响. **方法**: 用比浊法和放射免疫分析法. **结果**: 普鲁卡因胺 8.5, 34, 136 和 544 $\mu mol \cdot L^{-1}$有明显抑制作用. 抑制率分别为 45 % ± 37 %, 48 % ± 32 %, 88 % ± 23 %, 92 % ± 15 %和 53 % ± 24 %, 65 % ± 26 %, 90 % ± 6 %, 95 % ± 6 %. PA 的浓度与血小板聚集和 TXB_2 产生的抑制效力之间, 以及血小板聚集抑制率和 TXB_2 产生的抑制率之间均存在着正相关关系, 三者的线性方程和主要参数为: $\hat{Y} = 0.2075X - 4.9157$, $r = 0.9985$; $\hat{Y} = 0.9546X - 34.6724$, $r = 0.9921$; $\hat{Y} = 0.8202X + 19.7062$, $r = 0.9921$. **结论**: 普鲁卡因胺抑制凝血酶诱导血小板聚集和 TXB_2 产生.