BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Jul; **13** (4) : 330-333

Inhibitory effects of tetramethylpyrazine on platelets during cardiopulmonary bypass and arterial thrombus formation in dogs

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ABSTRACT The corrected platelet count at the end of cardiopulmonary bypass (CPB) in nontreated dogs fell to 56.7% of the pre-operative level, while in tetramethylpyrazine (TMP, 10 mg · kg^{-1}, iv)-treated dogs it fell to 82.8%. The number of α-granule membrane protein (GMP-140) which was expressed on the surface of activated platelets increased from the outset of CPB and reached the peak at the end of CPB in control dogs. But the number of GMP-140 molecules did not increase significantly throughout the operative procedure in TMP-treated dogs. In experimental thrombosis in dogs, the weight of the thrombus reduced to 23% of that in controls and the radioactivity ratio between the thrombus and blood was only 29% of that in controls at 4 h after iv injection of ^{125}I-SZ-51 (a monoclonal antibody against GMP-140). These results indicate that TMP inhibits the platelet activation during CPB and arterial thrombosis.

KEY WORDS tetramethylpyrazine; platelet activation; cardiopulmonary bypass; thrombosis; monoclonal antibodies; membrane proteins

Tetramethylpyrazine (ligustrazine, TMP), the main ingredient from *Liqusticum wallichii* Franch[1], is widely used for treatment of occlusive cerebral vascular diseases[2]. TMP could reduce the bioactivity of platelets and inhibit the rabbit platelet aggregation induced by ADP, which was related to the enchancement of membrane fluidity and electrophoretic mobility of platelets[3]. A recent study on the effects of TMP on mesenteric arterial muscle indicated that TMP inhibited the influx of Ca^{2+} as well as the release of intracellular Ca^{2-} [4]. Platelet activation and consumption during CPB leading to inadequate postoperative platelet number and function were recognized as the major contributors to bleeding after the operation[5]. The metabolites of platelets in plasma and the amount of GMP-140 expressed on the surface of activated platelets increased during CPB[6,7]. Thereafter, antiplatelet agent used for the preservation of platelet function was considered as an effective means of preventing the postbypass complications such as impaired hemostasis. Epoprostenol (prostacyclin) was reported to be an effective agent for inhibiting platelet activation during CPB[8]. In this study, we characterized the inhibitory effects of TMP on platelets in experimental CPB and arterial thrombosis, so as to study the inhibitory mechanism of TMP on platelets and thrombosis in dogs.

MATERIALS AND METHODS

TMP TMP 20 mg · ml^{-1} iv infusion solution in 2 ml ampoule was purchased from the 4th Pharmaceutical Factory of Beijing, lot № 880108.

Preparation of blood samples Mongrel dogs of either sex, weighing 12.5± *s* 2.5 kg, were anesthetized with iv sodium pentobarbital 30 mg · kg^{-1}. TMP (10 mg · kg^{-1}) was injected iv. The control dogs were injected with 0.9% NaCl. Bypass circuits contained a centrifuge pump and a bubble oxygenator (made in Xi'an Medical University, China). Heparin (300 IU · kg^{-1}) was given iv before cannulation of the aorta and right atrium. The CPB procedure lasted 90± 5 min in both groups. No blood nor platelet transfusion was given to any dog before or during the bypass procedure.

Received 1991 Mar 21 Accepted 1992 Mar 30

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Blood samples were obtained from the radial arterial catheter at 10 scheduled time intervals: before anesthesia, after heparinization, on-bypass 5, 15, 30, 60, and 90 min (end), post-bypass 15 min, after iv protamine (4.5 mg · kg^{-1}), and 2 h. The platelet count was performed with a light microscope. Hemodilution, caused by the iv infusion during CPB was taken into account by the dog's whole blood volume, obtained from weight and size tables. The blood anticoagulated with 2% EDTA-Na_2 (1·9, vol / vol) was fixed with an equal volume of 0.2% glutaraldehyde and 0.02% sodium azide in phosphate-buffered saline (PBS, pH 7.4).

Preparation and iodination of SZ-51 antibody SZ-51, a monoclonal antibody (McAb) which is specific against GMP-140 on the surface of activated platelets in both humans and dogs, was prepared in our institute[9-11]. The ascitic fluid induction was previously described[10]. There were 11 000 GMP-140 molecules expressed on the surface of thrombin-activated platelets while only 800 molecules on the surface of resting ones. Purified SZ-51 antibody was radiolabeled with $Na^{125}I$ (37 MBq, Beijing Institute of Atomic Energy, Chinese Academy of Sciences) by chloramine-T method[12]. The radiolabeled protein was separated from free ^{125}I by Sephadex G-25 column (Pharmacia). The specific radioactivity of the ^{125}I-protein was 200-400 Bq · ng^{-1} and more than 99% of the radioactivity was precipitated by 20% trichloroacetic acid. It was stored at 4℃ after the protein concentration was determined by the Folin phenol reagent of Lowry *et al* (1951).

Measurement of GMP-140 on platelet surface The measurement of GMP-140 molecules expressed on the surface of platelets was described previously[13].

Antithrombotic effect of TMP Dogs were anesthetized and the thrombus in the right femoral artery was produced as described previously[14]. Taking McAb specific for GMP-140 as a probe[11], TMP (10 mg · kg^{-1}) and ^{125}I-SZ-51 (11.1 MBq, 50 μg) were injected into the superficial veins of the left leg before operation and after the thrombus formation, respectively. The dogs were killed and the thrombus was taken out at 4 h after iv injection of the radiotracer. The thrombus was weighed and the radioactivity (dpm) was measured by a gamma ray counter. The radioactivity ratio between the thrombus and blood (T / B) was calculated as follows[14].

$$\text{T / B ratio} = \frac{\text{Thrombus dpm} \cdot \text{mg}^{-1}}{\text{Blood dpm} \cdot \text{mg}^{-1}}$$

RESULTS

Effect of TMP on platelet counts In the untreated dogs, the platelet count was (19.8 ± 2.3) × 10^{10} · L^{-1} before the experiment. Platelet counts corrected by hemodilution decreased to (16.4 ± 1.3) × 10^{10} · L^{-1} ($P<0.05$) at the outset of CPB and declined to (11.8 ± 0.3) × 10^{10} · L^{-1} ($P<0.01$) at the end of the procedure (Fig 1). The platelet count at the termination of CPB was only 56.7% of that before anesthesia. In contrast, platelet counts in the TMP-treated group were maintained almost constant throughout the CPB (Fig 1). The platelet count at the end of the CPB was 82.8% of that before anesthesia. Thereafter, the number of platelets during CPB remained quite stable following the TMP administration in dogs.

Effect of TMP on GMP-140 expression Five min after establishment of the bypass, the number of GMP-140 molecules on the platelet surface increased significantly as com-

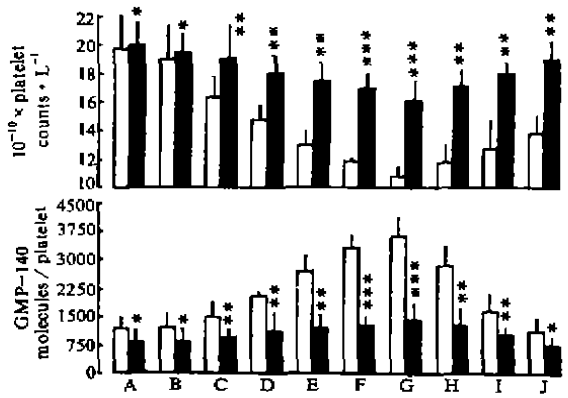

Fig 1. Platelet counts corrected for hemodilution and GMP-140 molecules on platelet surface during cardiopulmonary bypass in dogs. (□) **Control.** (■) **TMP 10 mg · kg^{-1}. A) before anesthesia; B) after heparinization; C) CPB 5 min; D) CPB 15 min; E) CPB 30 min; F) CPB 60 min; G) CPB 90 min; H) post-bypass 15 min, I) after iv protamine; J) post-bypass 2 h.** $n=5$, $\bar{x} \pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

pared with that before bypass and augmented thereafter in control dogs. The amount of GMP–140 molecules did not increase significantly in TMP–treated dogs throughout CPB (Fig 1).

Effect of TMP on thrombus formation in dogs In control dogs the weight of the thrombi was 108 ± 7 mg, while in TMP–treated dogs it was only 28 ± 5 mg at the 4 h after iv injection ^{125}I–SZ–51. This suggested that the arterial thrombosis was inhibited by TMP. In control group the radioactivity ratio between thrombus and blood (T / B) / mg of sample was 13 ± 2.9, while in TMP–treated group it was only 3.8 ± 0.7 at 4 h after iv injection of ^{125}I–SZ–51. This suggested that the expression of GMP–140 molecules within the thrombus was significantly inhibited.

DISCUSSION

In our study, the dog platelet aggregation induced by ADP at the final concentration of 8 μmol · L^{-1} was inhibited by TMP in a dose–dependent manner (IC_{50} = 20–30 mg · L^{-1}) (in press). Therefore, TMP 10 mg · kg^{-1} was chosen as a dosage for study *in vivo*. The expression of GMP–140 molecules on the platelet surface was inhibited by TMP throughout the experimental CPB in dogs. The results indicated that the release of α–granules of the dog platelets is inhibited and the function of platelets is preserved by TMP during CPB. Meanwhile, it could be concluded that the inhibitory effects of TMP on platelet releasing was one of the main causes for its inhibition on platelet aggregation *in vivo*. Owing to the short $T_{\frac{1}{2}}$ (29 min) of TMP, it is quickly eliminated from the circulation[15]. Thus, it was suggested that the inhibition of TMP on platelets might be transient and reversible. In view of the cheapness and easy availability of TMP, it is practicable to be tested in clinical use. However, the approapriate dosage of TMP needs clinical investigation.

In this study, the platelet count was stable in those dogs receiving TMP. This role of TMP was attributed to either the disaggregation of previously formed circulating platelet aggregates or the inhibition of platelet activation which resulted in lesser damage of platelets during CPB[5,6].

The inhibitory effect of TMP on arterial thrombosis suggested that the number of platelets participating in the thrombus formation was markedly decreased or the incomplete activation of platelets within the thrombus as compared to that in control dogs. This result indicates that TMP does inhibit the arterial thrombogenesis.

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四甲基吡嗪对犬体外循环中血小板及动脉血栓形成的抑制作用

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提要 犬体外转流结束，四甲基吡嗪(川芎嗪，TMP，iv 10 mg · kg^{-1})使血小板计数为转流前的82.8%，对照组为56.7%，血小板表面α颗粒膜蛋白分子数显著低于对照组($P<0.01$)。制备犬股动脉血栓模型4 h，TMP处理组离体血栓的重量为对照组的23%，而血栓与血液的放射活性比值仅为对照组的29%。提示TMP能抑制体外循环中血小板的活化及动脉血栓的形成。

关键词 四甲基吡嗪；血小板活化；心肺转流术；血栓形成；单克隆抗体；膜蛋白

BIBLID: ISSN 0253–9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Jul; **13** (4) : 333–337

Global depletion of myocardial norepinephrine and ATP after left coronary artery occlusion in rats[1]

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ABSTRACT After ligation of the left coronary artery in rats, myocardial norepinephrine (NE) and ATP depletions in both infarcted (IZ) and non–infarcted zone (NIZ) were studied. In IZ, the depletions of NE and ATP were biphasic and the depleting rate constants were found to be $K_1 = 0.71$ h^{-1} and $K_2 = 0.015$ h^{-1} for NE, and $K_1' = 0.52$ h^{-1} and $K_2' = 0.016$ h^{-1} for ATP. In NIZ, the depletion of NE was monophasic, slowly progressive, and quite durable with rate constant $K_3 = 0.018$ h^{-1}; The depletion of ATP was transient. Propranolol (Pro) and verapamil (Ver) were beneficial but only partly effective against NE and ATP depletions.

KEY WORDS myocardial infarction; propranolol; verapamil; norepinephrine; epinephrine; adenosine monophosphate; adenosine biphosphate; adenosine triphosphate

Received 1991 Jan 21 Accepted 1992 May 6
[1] Supported by a grant from the Office of Science & Technology, Bureau of Health, Jiangsu Province, China.

The release of norepinephrine (NE) would