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A selective serotonin reuptake inhibitor, citalopram, inhibits collagen-induced platelet aggregation and activation

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ABSTRACT

Clinical depression is a significant risk factor for cardiovascular diseases and confers an increased risk of mortality. Increased platelet reactivity may predispose depressed patients to cardiovascular diseases. The antidepressants selective serotonin reuptake inhibitors (SSRIs) have been found to have cardioprotective effects probably via the attenuation of platelet activation independently in addition to treatment of depression itself. However, the characters of the inhibitory effect of SSRIs on platelets remain largely unknown. Here we show that an SSRI, citalopram, specifically inhibited collagen-induced platelet aggregation. Citalopram, however, revealed only little inhibitory effect on platelet aggregation induced by thrombin, U46619, and ionomycin, and failed to inhibit reversible platelet aggregation induced by adenosine diphosphate with fibrinogen. Collagen-induced of $\alpha_{IIb}\beta_3$ integrin activation in platelets under a static condition was not influenced by citalopram. Citalopram inhibited convulxin-induced platelet aggregation and $\alpha_{IIb}\beta_3$ integrin activation. In the experiments with fibrinogen-induced aggregation in elastase-treated platelets, citalopram inhibited only collagen-induced $\alpha_{IIb}\beta_3$ activation but not the binding activities between activated $\alpha_{IIb}\beta_3$ integrin and fibrinogen. Moreover, citalopram inhibited α-granule and dense granule secretion from platelets in response to collagen, as determined by a reduced expression of P-selectin and adenosine triphosphate release, respectively. In addition, collagen-induced thromboxane A_2 release in platelets was attenuated by citalopram pretreatment. These findings might specify the mechanisms of inhibitory effects of citalopram on collagen mediated platelet activation and aggregation, and further support the cardioprotective effect of SSRIs.

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Introduction

Numerous studies have provided substantial evidence that depression is associated both with the development and prognosis of cardiovascular disease [1,2]. Among multiple mechanisms involving in the relationship between depression and cardiovascular diseases, platelet hyperactivity is reported to be a potential biologic abnormality associated with depression [3–5]. The importance of platelet activation and its inhibition is clearly supported by the clinical benefits of using the antiplatelet agents for patients of cardiovascular disease [6]. Interestingly, depression treatments could improve cardiovascular prognosis in patients with coronary artery disease [7]. The selective serotonin reuptake inhibitors (SSRIs) are well established antidepressants with safety profile for patients with heart disease [8,9]. Moreover, treatment with SSRIs could directly exert inhibitory effects on platelet reactivity [10].

Depression is characterized by serotonin dysfunction in the brain, which could be modulated by recycling through a reuptake and repackaging mechanism [11]. The concept of blockade of the presynaptic reuptake of serotoninin in the brain has led to the development of SSRIs [12], which are now the most widely used antidepressant to treat this condition. In human body, more than 99% of serotonin is stored in platelets [13]. Platelets share biochemistry similarity with central serotonergic neurons in the uptake, storage, and metabolism of serotonin, and have been used as a powerful surrogate to study brain serotonergic synaptosomes [14]. Consistent to the findings from brains, SSRIs also block the reuptake of serotonin into platelet dense granule [15]. It has been reported that a prolonged intake of SSRIs in high doses leads to a significant depletion of intra-platelet serotonin and, subsequently, reduces platelet activation [9,16,17].

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; c7E3, chimeric 7E3 antibody; GP, glycoprotein; PGE_1, prostaglandin E_1; PRP, platelet-rich plasma; SSRI, selective serotonin reuptake inhibitor; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2.

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Platelets respond at the site of vascular injury, where successive phases are involved including an adhesion of glycoprotein Ib (GPIb) and glycoprotein VI (GPVI) to von Willebrand factor and collagen, respectively. The response then triggers the release of granules and thromboxane A_2 (TxA_2) and the activation of integrin $\alpha_{IIb}\beta_3$, which binds to fibrinogen and allows platelet aggregation [18]. Meanwhile, platelet serotonin is released from dense granule during activation. Notably, serotonin is merely a weak platelet agonist that needs adenosine diphosphate (ADP) or epinephrine to enhance its effect. The platelet-release serotonin has an established role in aiding in hemostasis by a regulation of vasoconstriction or dilation rather than by the direct influence of platelet aggregation [13]. Given that serotonin is a weak agonist for the platelet aggregation, it is too simple to attribute the inhibitory effect of SSRIs on platelet aggregation to the depletion of intra-platelet serotonin after a prolong treatment of SSRIs. Moreover, the role of SSRIs involved in the multiple phases of the response of platelets to agonists remains largely unknown. In this study, we found that citalopram, one of the widely used SSRIs, specifically inhibits collagen-induced platelet aggregation in the experiments with isolated platelet preparation. We further characterized the inhibitory effects of citalopram on the granule secretion and TxA_2 released by platelets in response to collagen challenges.

Methods and materials

Antibodies and reagents

Fluorescein isothiocyanate (FITC)-conjugated mouse anti-human $\alpha_{IIb}\beta_3$ integrin antibody PAC-1 and FITC-conjugated mouse monoclonal anti-human P-selectin antibody AK-4 were purchased from Becton Dickinson (San Diego, CA, USA). Anti-human integrin $\alpha_{IIb}\beta_3$ chimeric 7E3 antibody (c7E, ReoPro) was kindly supplied by Eli Lilly, Taiwan. All antibodies were used at optimal concentrations, as determined by titration.

Citalopram, human thrombin, adenosine diphosphate (ADP), ionomycin, elastase (type IV), bovine serum albumin (BSA), prostaglandin E_1 (PGE_1) and collagen (type I, bovine Achilles tendon) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Convulxin was purchased from LOXO (Dossenheim, Germany). U46619 was from Cayman Chemical (Ann Arbor, MI, USA). Heparin was from LEO Pharma (Ballerup, Denmark). BCECF-AM was from Molecular Probes (Leiden, The Netherlands). Other chemicals were purchased from Sigma-Aldrich or Wako (Osaka, Japan).

Preparation of platelet suspension and elastase-treated platelet suspension

Human platelets were purified as previously described [19]. This study was approved by the Committee of Ethics of China Medical University Hospital. In brief, blood from healthy donors was collected by venipuncture into acid-citrate-dextrose (9:1) and centrifuged at $200\times g$ for 20 min at 25 °C to prepare platelet-rich plasma (PRP). PRP then was firstly washed with modified Tyrode's solution (NaH_2PO_4, 0.4 mM; NaCl, 136.9 mM, KCl 2.7 mM; $NaHCO_3$, 11.9 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM; 0.35% BSA and 0.1% glucose) containing heparin (7 U/ml) and PGE_1 (6 nM), and centrifuged at $500\times g$ for 15 min at 25 °C. After descanting the supernatant, pellet was then washed twice with modified Tyrode's solution containing heparin and PGE_1. Finally, washed platelet were resuspended to a final concentration of 3×10^8 platelets/ml in Tyrode's solution containing 0.35% BSA and incubated at 37 °C for use.

Elastase-treated platelets were prepared as previously described by Komecki [20]. Briefly, human platelet suspensions were incubated with elastase (10 U/ml) for 1 h at 37 °C. Platelets were then washed three times by centrifugation and resuspended in modified Tyrode's solution.

Platelet aggregation assay

Platelet aggregation was measured with an aggregometer (Payton Scientific, Buffalo, NY, USA) as previously described [21]. Briefly, platelet suspension at concentration of 3.5×10^7 per millimeter was applied to the aggregometer and stirring was initiated at 900 rpm for 1 min at 37 °C with a small magnetic bar. Then various concentrations of indicated citalopram were added and incubated for 3 min followed by adding proaggregatory substance thrombin (1 U/ml), U46619 (10 μM), collagen (10 μg/ml), ADP (20 μM), ionomycin (10 μM) or convulxin (200 ng/ml). We used PowerLab 8/SP (ADInsturments, Sydney, Australia) to analyze the extent of platelet aggregation that was continuously monitored for 8 min by turbidimetry and expressed as increase of light transmission.

Flow cytometry

The expression of activated integrin $\alpha_{IIb}\beta_3$ and P-selectin was performed by using cytofluorimetric analysis. All reactions were performed with 100 μl total volume containing 1×10^6 isolated platelets. In some experimental conditions, c7E3 (10 μg/ml) was added to inhibit platelet aggregation. After pretreatment with indicated concentrations of vehicle solution, citalopram, c7E3 or PGE_1 for 3 min at 37 °C, isolated platelets were stimulated by the agonist collagen (10 μg/ml) or convulxin (200 ng/ml) and then incubated for 10 min. After extensive washes, platelets were labeled with monoclonal antibodies raised against P-selectin (AK-4) or activated integrin $\alpha_{IIb}\beta_3$ (PAC-1) at room temperature for 30 min. Cells then washed, resuspended in phosphate buffer saline, and analyzed immediately by FACSCanto (Becton Dickinson, USA).

Platelet adhesion assay

Ninety-six flat-bottom well microliter plates were coated with fibrillar collagen or fibrinogen (50 μg/ml) at 4 °C overnight. Wells were then blocked with 1% BSA in PBS for 1 h at 37 °C. Platelets were labeled with fluorescent dye BCECF-AM for 30 min. After a brief wash, platelets were allowed to seed with various concentration of citalopram for 1 h at 37 °C. At the end of the incubation, non-adherent cells were removed by a brief wash with PBS. Attached platelets were read by a Cytofluor microplate reader with fluorescence excitation and emission wavelength at 485 nm and 530 nm, respectively. Adhesion of platelets was quantified as the percentage of fluorescence intensity of control platelets.

Measurement of ATP release

A luciferin-lucifease detection system, adenosine triphosphate (ATP) Bioluminescent Assay Kit (Sigma-Aldrich), was used to quantify ATP release of platelets. The platelets were pretreated with various concentrations of citalopram or with vehicle solution for 3 min at 37 °C, and stimulated with collagen (10 μg/ml) under a stirring condition. In some experimental conditions, c7E3 (10 μg/ml) was added to inhibit platelet aggregation. At indicated time points the samples were centrifugated at 14000 cpm for 30 sec at room temperature. After adding an equal volume of luciferin-luciferase solution to the supernatant, chemoluminescence was determined immediately using a luminometer (Modulus ™; Turner BioSystems, Sunnyvale, CA, USA).

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