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Regular Article

The anti-aggregation effects of ondansetron on platelets involve IP3 signaling and MAP kinase pathway, but not 5-HT3-dependent pathway

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ABSTRACT

Ondansetron is a 5-HT3 receptor antagonist with potent antiemetic, analgesic, and antiphlogistic effects. Literature concerning 5-HT3 antagonists on platelets is limited. In this report we examined the pharmacological effects of ondansetron on human washed platelets. Platelet aggregation induced by thrombin (0.1 U/mL), collagen (2 μg/mL), arachidonic acid (0.5 mM), ADP (10 μM), or U46619 (2 μM) was observed. The effects of ondansetron on platelet aggregation and ATP release were investigated at different concentrations. Cytosolic Ca^{2+} influx concentration, TXB2, IP3, and the levels of cAMP and cGMP were monitored, and flow cytometric analysis and immunoblotting were performed to investigate downstream signaling components. Our results showed that ondansetron, in a concentration-dependent manner, inhibited agonist-induced platelet aggregation. At 75 μM, ondansetron significantly attenuated intracellular Ca^{2+} mobilization, thromboxane B2 formation, and ATP release by human washed platelets activated by thrombin, collagen, or U46619, whereas it only partially attenuated arachidonic acid-driven platelet activation. Administration of ondansetron resulted in attenuated IP3 production in the washed platelets stimulated by thrombin, as determined by reduced IP1 levels, as well as diminished p38 and ERK2 phosphorylation in response to thrombin. No effect of ondansetron on the levels of either cAMP or cGMP in washed platelets was observed. Furthermore, ondansetron-mediated inhibition of platelet aggregation was not impacted by SR 57227A, the 5-HT3 agonist. Thus, rather than involving the 5-HT3-dependent pathway, the negative effect of ondansetron on platelet aggregation is instead manifested through the attenuation of agonist-induced IP3 production and MAPK (p38 and ERK2) phosphorylation that results in suppressed intracellular Ca^{2+} mobilization, TXB2 formation, and ATP release.

Introduction

Ondansetron, 1,2,3,9-Tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one (Fig. 1), is a selective antagonist of the serotonin 5-hydroxytryptamine 3 (5-HT3) receptor and a known potent antiemetic in clinical application that has been used extensively in patients to effectively treat nausea/vomiting due to postoperative, chemotherapy and radiation therapy [1,2]. For the indication of prevention of chemotherapy-induced nausea and vomiting (CINV), three doses of 0.15 mg/kg ondansetron by IV is recommended. For the prevention of postoperative induced nausea and vomiting (PONV), a single dose of 0.1 mg/kg is recommended in patients [3].

We have recently promulgated the putative role of ondansetron on the hepatic injury and trauma-hemorrhage as well as its modulating effect on pro-inflammatory mediators, such as interleukin (IL)-6, intercellular adhesion molecule (ICAM)-1, cytokine-induced neutrophil chemoattractant (CINC)-1, and CINC-3, via a p38 mitogen-activated protein kinase (MAPK) pathway [4]. Nevertheless, the effect of ondansetron on platelet functions remains to be defined. Mitogen-activated protein kinase has been reported to regulate inflammatory responses to various stimuli in different cell types [5,6]. Furthermore,

Abbreviations: SIR2, silent information regulator 2; TXB2, thromboxane B2; AA, arachidonic acid; 5-HT3, 5-hydroxytryptamine 3, histone deacetylase; IP, inositol phosphate; IP3, inositol 1,4,5-triphosphate; MAPK, mitogen-activated protein kinases; ERK, extra cellular regulated protein kinases; FITC, fluorescein isothiocyanate; CEPI-CT, Collagen/epinephrine closure time; CADP-CT, collagen/ADP closure time; PGE1, prostaglandin E1; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; SNP, sodium nitroprusside; IBMX, 3-isobutyl-1-methylxanthine; BSA, bovine serum albumin; EDTA, ethylenediamine tetraacetic acid; DMSO, dimethylsulfoxide; PRP, platelet-rich plasma; PPP, platelet- poor plasma; AC, adenylate cyclase; PDE, phosphodiesterase.

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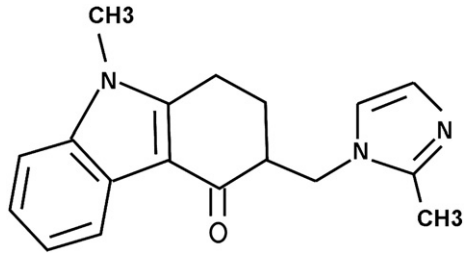

Fig. 1. Chemical structure of ondansetron.

MAPK also plays an important role in platelet adhesion and activation [7,8]. Meanwhile, previous studies showed an increased presentation of 5-HT receptors on the surface of activated platelet [9–11]. Recent reports confirmed by Western blot the expression of the 5-HT3A receptor on unstimulated as well as ADP- and TRAP-stimulated platelets [12]. Observations have also been made that 5-HT3 receptor antagonists possess both analgesic and antiphlogistic effects, the latter effect hinting at the presence of 5-HT3 receptors in various cell types involved in inflammation, including platelets [13,14]. Taken together, these observations suggest that, at the aforementioned clinically relevant concentration, ondansetron may regulate platelet activities, in addition to modulating inflammatory responses. However, little is known about the pharmacological functions of 5-HT3 in platelets, and no data is available to provide insight on the impact of 5-HT3 receptor antagonists on platelets.

In this study, we will systematically examine the influence of ondansetron on the activities and functions of normal human platelets to characterize its potential effect on platelet aggregation and elucidate the underlying mechanisms for application on future studies of 5-HT3 inhibitors.

Materials and Methods

Blood Sampling

Healthy volunteers without history of hematological diseases such as platelet or coagulation disorder, and without taking medication that might affect hematological function, were recruited for this study. Each volunteer first signed the inform consent, and then a 30-mL amount of blood was drawn from the volunteer. All the experimental procedures and protocols were approved by the Institutional Review Board of Chang Gung Memorial Hospital, Linkou Branch (Gueishan, Taoyuan, Taiwan ROC).

Chemicals

All the chemicals used in these experiments were of the highest purity grade available from each supplier. Zofran (ondansetron hydrochloride) was bought from GlaxoSmithKline plc (Brentford, Middlesex, UK). Ondansetron and granisetron were obtained from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Thrombin, collagen, arachidonic acid, ATP standard, and Chrono-Lume were purchased from Chrono-Log Co. (PA, USA). ADP, U46619, serotonin, SR 57227A, indomethacin, apyrase, prostaglandin E1 (PGE1), sodium nitroprusside (SNP) 3-isobutyl-1-methylxanthine (IBMX), bovine serum albumin (BSA), Ethylenediamine tetraacetic acid (EDTA), and dimethylsulfoxide (DMSO) were also obtained from the Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Fluo-3 AM was bought from Molecular Probes (Eugene, Oregon, USA). Fluorescein isothiocyanate (FITC) and CD62P (P-selectin) antibody were obtained from BD Biosciences (San. Jose, CA, USA). Phospho-p38 MAPK (Thr180/Tyr182) and total p38 MAPK antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). Cyclic AMP, cyclic GMP, and thromboxane B2 EIA kits were purchased from Cayman Chemical (Ann Arbor, USA). Collagen/epinephrine (CEPI) and collagen/ADP (CADP) cartridges were purchased from Dade-Behring, Inc. (Newark, DE, USA). Ondansetron was dissolved in normal saline (0.9%) and stored at −20 °C until use.

Preparation of Platelets

30-mL Blood samples were drawn from healthy volunteers who had not taken any regimens within two weeks prior to blood-drawing. Each sample of drawn blood was collected in a 50-mL sample tube containing sodium citrate as anticoagulant at the final concentration of 3.15% (1:9 v/v). For preparation of platelet-rich plasma (PRP), blood samples were centrifuged at 300 ×*g* for 10 min, and the resultant supernatants were collected. The remaining lower layer of the centrifuged samples was further subjected to a second centrifugation at 850 ×*g* for 15 min, and the resultant supernatants were harvested as platelet-poor plasma (PPP). To prepare washed platelets, PRP was first centrifuged at 1,050 ×*g* for 6 min at room temperature. The platelet pellets were then washed with modified Tyrode-HEPES buffer (129 mmol/L NaCl, 2.8 mmol/L KCl, 8.9 mmol/L $NaHCO_3$, 0.8 mmol/L $MgCl_2$, 0.8 mmol/L KH_2PO_4, 2 mmol/L EGTA, 5.6 mmol/L glucose, 10 mmol/L HEPES, 0.35% BSA, pH 7.4) and then centrifuged again at 1050 ×*g* for another 6 min. Finally, the washed platelets were gently resuspended in Tyrode-HEPES buffer (129 mmol/l NaCl, 2.8 mmol/l KCl, 8.9 mmol/l $NaHCO_3$, 0.8 mmol/l $MgCl_2$, 0.8 mmol/l KH_2PO_4, 1 mmol/l $CaCl_2$, 5.6 mmol/l glucose, 10 mmol/l Hepes, 0.35% BSA and 0.1 U/mL apyrase, pH 7.4). The platelet concentrations were counted by Drew Hemavet 950FS® analyzer (Drew Scientific, Oxford, CT, USA) and adjusted to a concentration of 2×10^8 platelets/mL.

PFA-100 Analyzer Studies

To study the clot formation under the dynamic situation, we deployed the PFA-100 Analyzer (Dade Behring) for the study. Citrated blood samples were first allowed to sit at room temperature for 10 min. Three minutes before analysis, the samples were then incubated with Zofran (ondansetron hydrochloride) and granisetron at 50, 75 and 100 μM or vehicle. All blood samples were tested for closure times with collagen/epinephrine (CEPI) and collagen/ADP (CADP) cartridges according to the manufacturer's instructions. The time required to obtain full occlusion of the aperture is defined as the collagen/ADP closure time (CADP-CT) or collagen/epinephrine closure time (CEPI-CT). All the samples were tested no later than one hour after blood drawing, and the upper limit of the closure time was set to 300 seconds by default.

Platelet Aggregation and ATP Release Reaction

Both the platelet aggregation and release of ATP from platelets were simultaneously measured by Lumi-aggregometer Model 560 (Chrono-Log, USA). Washed platelets (or PRP) were incubated and stabilized at 37 °C in an aggregometry sample tube with stirring at 1000 rpm for 1 minute before testing. The samples were further pretreated with ondansetron (25, 50, and 75 μM) or vehicle for 3 min, and then platelet aggregation was induced by addition of collagen (2 μg/mL), AA (0.5 mM), thrombin (0.1 U/mL), ADP (10 μM), or U46689 (2 μM). The resulting aggregations measured as the change of light transmission were recorded for 10 min. The extent of platelet aggregation is expressed as percentage of light transmission (% Light Transmission) in platelet-free medium; as platelet aggregation increases, the turbidity of sample drops due to clearance of platelet in suspension, and % Light Transmission increases accordingly. The ATP released from platelets was detected by the Chrono Lume luciferase reagent according to the manufacturer's directions with gain control set at 0.005×. The final sample volume was made up to 0.5 mL for measurements.