

Video Article

Turbidimetry on Human Washed Platelets: The Effect of the Pannexin1-inhibitor Brilliant Blue FCF on Collagen-induced Aggregation

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Abstract

Turbidimetry is a laboratory technique that is applied to measure the aggregation of platelets suspended in either plasma (platelet-rich plasma, PRP) or in buffer (washed platelets), by the use of one or a combination of agonists. The use of washed platelets separated from their plasma environment and in the absence of anticoagulants allows for studying intrinsic platelet properties. Among the large panel of agonists, arachidonic acid (AA), adenosine di-phosphate (ADP), thrombin and collagen are the most frequently used. The aggregation response is quantified by measuring the relative optical density (OD) over time of platelet suspension under continuous stirring. Platelets in homogeneous suspension limit the passage of light after the addition of an agonist, platelet shape change occurs producing a small transitory increase in OD. Following this initial activation step, platelet clots form gradually, allowing the passage of light through the suspension as a result of decreased OD. The aggregation process is ultimately expressed as a percentage, compared to the OD of platelet-poor plasma or buffer. Rigorous calibration is thus essential at the beginning of each experiment. As a general rule: calibration to 0% is set by measuring the OD of a non-stimulated platelet suspension while measuring the OD of the suspension medium containing no platelets represents a value of 100%. Platelet aggregation is generally visualized as a real-time aggregation curve. Turbidimetry is one of the most commonly used laboratory techniques for the investigation of platelet function and is considered as the historical gold standard and used for the development of new pharmaceutical agents aimed at inhibiting platelet aggregation. Here, we describe detailed protocols for 1) preparation of human washed platelets and 2) turbidimetric analysis of collagen-induced aggregation of human washed platelets pretreated with the food dye Brilliant Blue FCF that was recently identified as an inhibitor of Pannexin1 (Panx1) channels.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55525/>

Introduction

Platelets are crucial components of blood and their main function-together with coagulation factors-is to stop bleeding after blood vessel injury. Platelets are small (2-3 μm) anuclear fragments derived from megakaryocytes of the bone marrow¹. Platelets circulate in non-activated state, during which they appear as lens-shaped structures. Upon interruption of the endothelium, platelets gather to the site of blood vessel injury to plug the hole, a process called primary hemostasis. Initially, platelets attach to sub-endothelial molecules, such as collagen and von Willebrand factor, that are exposed as a result of the injury-adhesion step². Then, they change shape and secrete chemical messengers-activation step. Finally, they connect to each other by bridging receptors-aggregation step. Primary hemostasis is followed by a secondary process involving activation of the coagulation cascade with fibrin deposition, which stabilizes the initial thrombus².

Acute ischemic events such as myocardial infarction³ often result from thrombi that form because of physical disruption (rupture) of an atherosclerotic plaque. Current anti-platelet drugs are the cornerstone of the treatment of this widespread disease but their clinical benefit is limited by an increased risk for bleeding. The most prescribed drugs in cardiovascular patients, aspirin and anti-P2Y₁₂ compounds, target the thromboxane A₂ and the ADP pathways⁴, respectively, which are the major pathways leading to platelet activation. However, innovative research towards new targets that would optimally balance antithrombotic effects and haemorrhagic risk is still necessary.

From the 1960s⁵ to today, turbidimetric aggregometry has played a crucial role in research, enhancing our knowledge of platelet reactivity and in the monitoring of the potency of anti-thrombotic reagents in humans. Turbidimetry was initially applied to PRP extracted from blood samples. Indeed, blood collection performed in tubes containing citrate allows fast and large production of PRP without having any effect on platelet integrity and function. However, the short-term stability (about 3 h) of PRP and the remaining plasmatic enzymes, such as thrombin, and the low calcium concentration associated with potentially artefactual aggregation profiles are of major inconvenience for the use of PRP. An important step forward has been the development of a method for platelet isolation with additional centrifugation and washing steps⁶. In short, PRP is isolated from whole blood collected on acid-citrate-dextrose (ACD) and platelets are isolated after serial centrifugation steps before being resuspended in an iso-osmotic phosphate buffer (Tyrode's buffer) containing glucose, human serum albumin and divalent cations (Ca^{2+} and Mg^{2+}). To avoid changes in platelet reactivity, the pH of Tyrode's buffer is carefully kept at 7.35-7.4. Moreover, undesired activation of platelets is

prevented by adding prostacyclin (PGI_2) before some centrifugation steps. Finally, addition of apyrase prevents washed platelets from becoming resistant against the action of ATP/ADP. The resulting platelet suspension lacks coagulant factors and the stability of platelets is increased by at least two-fold as compared to PRP solutions. In addition, the fact that platelets are inactive but intact warrants the reproducibility of turbidimetric measurements and provides the ability to study the action of agonists or antagonists of platelet aggregation in an optimal way.

Using this method, we have shown in a recent study that inhibiting the formation of Panx1 channels by a genetic approach (knock-out mice) or decreasing Panx1 channel activity by pharmacological approaches reduced collagen-induced platelet aggregation⁷. Panx1 forms ATP-release channels, which are ubiquitously expressed in many cell types including human platelets^{7,8}. In fact, we demonstrated by turbidimetry on human washed platelets that a 7 min preincubation with a panel of more-or-less specific chemical blockers (probenecid, mefloquine and ¹⁰Panx1 peptides) prior to the addition of various agonists, inhibited specifically collagen-induced platelet aggregation while platelet responses to AA and ADP were not affected. We demonstrated that ATP release through Panx1 channels specifically interferes in the GPVI signaling pathway leading to collagen-induced aggregation. Interestingly, multiple FDA-approved compounds with applications in other diseases (probenecid, mefloquine) affect the activity of Panx1 channels in platelets. On one hand, this opens new therapeutic perspectives to selectively modify platelet reactivity. On the other hand, one should consider potential secondary effects of these compounds. In this context, the safe food dye Brilliant Blue FCF used in multiple candies and energy drinks has been described as a selective inhibitor of Panx1⁹. We describe here a protocol for the isolation of human washed platelets and turbidimetric measurements of platelet aggregation adapted to investigate the effect of the Brilliant Blue FCF dye as an antagonist of platelet aggregation.

Protocol

Five unrelated healthy volunteers were recruited for blood sampling for platelet isolation and aggregation tests. Written informed consent was obtained and the protocol was approved by the Central Ethics Committee of the Geneva University Hospitals. All volunteers certified to be healthy and to have not taken any platelet-interfering drugs during at least the 10 days preceding the experiments.

1. Buffer Preparation for Human Blood Collection and Washed Platelet Isolation

1. Prepare a 100 mL aqueous solution of acid-citrate-dextrose (ACD) by dissolving 1.4 g citric acid monohydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$, 66.6 mM), 2.5 g trisodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2 \text{H}_2\text{O}$, 85 mM) and 2 g of anhydrous D(+)-glucose. The pH of the solution is about 4.5.
2. **Prepare stock solutions for Tyrode's buffer as follows**
 1. Prepare stock solution 1 by dissolving 80 g NaCl, 2 g KCl, 10 g NaHCO_3 and 0.58 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 500 mL of distilled H_2O . The respective final concentrations are 2.73 M, 53.6 mM, 238 mM and 8.4 mM. Keep the solution at 4 °C.
 2. Prepare stock solution 2 by dissolving 10.15 g $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ (100 mM) in 500 mL distilled H_2O . Keep solution at 4 °C.
 3. Prepare stock solution 3 by dissolving 10.95 g (100 mM) $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$ in 500 mL distilled H_2O . Keep solution at 4 °C.
3. Prepare Tyrode's buffer by diluting 2.5 mL of stock solution 1 in a final volume of 50 mL with distilled H_2O . This corresponds to final concentrations of 136.5 mM NaCl, 2.68 mM KCl, 11.9 mM NaHCO_3 and 0.42 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. Adjust the pH to 7.35 and sterilize by filtering with 0.22- μm filters.
4. **Prepare Tyrode's albumin 0.35% buffer (TA buffer⁷) by diluting 5 mL of stock solution 1, 1 mL of stock solution 2, 2 mL of stock solution 3, 0.5 mL 1M HEPES, 1.8 mL of 200 g/L human serum albumin and 0.1 g of anhydrous D(+)-glucose in a final volume of 100 mL distilled H_2O .**
 1. Adjust the pH to 7.35 with 1N HCl and set the osmolality to 295 mOsm/L by adding distilled H_2O (10% of total volume). Final concentrations in this solution are: 124 mM NaCl, 2.44 mM KCl, 10.82 mM NaHCO_3 , 0.38 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.91 mM $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$, 1.82 mM $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$. Keep TA buffer at 37 °C during the whole experiment.

2. Blood Collection

1. **Collect 45-50 mL of venous blood, from the antecubital vein using a 19 G needle and no or low tourniquet, into 50 mL tubes containing ACD anticoagulant (1 volume ACD for 6 volumes of blood). Discard the first 1-2 mL of blood to avoid the presence of thrombin and tissue factor.**
 1. After collection, mix the blood with the ACD by gently inverting the tube. Incubate the sample for 10 min at 37 °C.

3. Preparation of Human Washed Platelets

1. Pre-heat the centrifuge to 37 °C. All centrifugation steps below are performed at this temperature.
2. Dispatch the collected blood into 15 mL tubes (5 mL per tube) and centrifuge at 250 x g for 13 min to obtain PRP.
NOTE: This centrifugation step results in the production of three layers in the sample: 1) The upper layer, composed of plasma, platelets, and a small fraction of white blood cells. 2) The intermediate layer, a portion rich in white blood cells. 3) The bottom layer, which is essentially composed of red blood cells.
3. Collect the PRP by pipetting the upper layer carefully into a new 15 mL tube to maximally prevent contamination with red and white blood cells, and incubate for 10 min at 37 °C.
4. Centrifuge the PRP at 2,200 x g for 12 min (for 5 mL PRP).
NOTE: This centrifugation step should be performed with low brake or without brake.
5. Remove the supernatant (platelet-poor plasma), and carefully resuspend the pellet with 10 mL of TA buffer containing 2 $\mu\text{L}/\text{mL}$ of heparin (5,000 U/mL) and 2.5 $\mu\text{L}/\text{mL}$ of 25 μM PGI_2 using a plastic Pasteur pipet. Incubate for 10 min at 37 °C.
6. Add 2.5 $\mu\text{L}/\text{mL}$ of 25 μM PGI_2 and centrifuge for 8 min at 1,900 x g (with low brake or without brake).

7. Remove the supernatant and resuspend the pellet with 5 mL TA buffer containing 2.5 $\mu\text{L/mL}$ of 25 μM PGI_2 using a plastic Pasteur pipet. Incubate for 10 min at 37 $^\circ\text{C}$.
8. During the incubation period, pipet 150 μL of the platelet suspension into a 1.5 mL tube and count platelets, using an automatized cell counter (that detects the size of blood cells by measuring the changes in direct-current resistance).
9. After the 10 min incubation, add 2.5 $\mu\text{L/mL}$ of 25 μM PGI_2 to the platelet suspension and immediately centrifuge at 1,900 $\times g$ for 8 min.
10. Remove the supernatant and resuspend the pellet to a concentration of 250,000 platelets/ μL with an adequate volume of TA buffer (*i.e.* if the cell count is 500,000 per μL , resuspend in 10 mL TA buffer) containing 32 $\mu\text{L/mL}$ of apyrase at 0.01 U/mL (final concentration 0.32 U/mL).
NOTE: High concentration of apyrase is used to avoid the desensitization of P2X1 receptors induced by spontaneous secretion of ATP^{10,11} in absence of agonists. This is important because collagen-induced responses are induced by fast paracrine/autocrine activation of P2X1 by ATP released from activated platelets. If the platelet signaling pathway does not critically require preservation of P2X1 function, use 0.02 U/mL apyrase. Several studies (reviewed in Mahaut-Smith *et al.*¹⁰) demonstrated that 0.02 U/mL apyrase avoids ADP receptor P2Y1 desensitization with negligible P2X1 responses.
11. Incubate the cell suspension for at least 30 min at 37 $^\circ\text{C}$ before performing the aggregometric measurements. The preparation is stable for 5 to 8 h.

4. Aggregometry

1. Prepare fibrinogen (56 mg/mL) in Tyrode's buffer.
2. Pipet 260 μL of platelet suspension into glass cuvettes (**Figure 1A**; left cuvette) containing 10 μL of fibrinogen (56 mg/mL) and a magnetic stirring rod, then incubate the suspension for 2 - 3 min at 37 $^\circ\text{C}$ in incubation wells present in the aggregometer (**Figure 1B and 1C**).
3. Pre-incubate with the P2X1 inhibitor Brilliant Blue FCF by adding 10 μL of a 2.8 mM or 28 mM stock solution (final concentration 100 μM and 1 mM, respectively) for 7 min at 37 $^\circ\text{C}$.
4. **Calibrate the aggregometer to an assumptive 100% aggregation value by measuring the OD of a cuvette containing 10 μL fibrinogen (56 mg/mL), 10 μL Brilliant Blue FCF (2.8 mM or 28 mM) and TA buffer without platelets.**
 1. Place the cuvette in an aggregation well under automatic stirring and press the corresponding button on the keyboard of the computer linked to the aggregometer (*i.e.* press F1 if aggregation well 1 is used).
NOTE: This experiment described below includes Brilliant Blue FCF. The compound used for calibration has to be adjusted to the experimental condition.
5. **Calibrate the aggregometer to an assumptive 0% aggregation value by using the same platelet sample that will be used for the experiment under automatic stirring.**
 1. Place the cuvette in the aggregation well and press the corresponding button on the keyboard of the computer linked to the aggregometer. Wait for about 20-30 s before proceeding. This delay serves to assure that no aggregation happens before adding the agonists.
NOTE: As any difference in platelet number may have an effect on the measured OD, the 0% calibration step needs to be repeated for each individual measurement.
6. Add 20 μL of desired agonist, such as 15 $\mu\text{g/mL}$ collagen (1 $\mu\text{g/mL}$ final) or 1.125 mM arachidonic acid (75 μM final), into the cuvette. Immediately start the recording under continuous automatic stirring by pressing the corresponding button on the keyboard of the computer linked to the aggregometer.
NOTE: The addition of the agonist induces platelet activation. Platelet aggregates can clearly be distinguished in the glass cuvette at the end of the experiment (**Figure 1A**; right cuvette).
7. The recording automatically stops after 6 min. At this point, save the data by clicking on the save icon of the computer.
NOTE: The calculation of the rate of aggregation is performed by the computer, which expresses the end results of the aggregation process as a percentage.
8. **Analyze the data.**
 1. For additional extensive information on protocols for the preparation of washed platelets suspensions and turbidimetric measurement of platelet aggregation, refer to other papers authored by experts in the field^{12,13}.

Representative Results

The aggregometer software automatically produces the aggregation curves and gives the values for maximal aggregation in percentage. The values can be copied to a data analysis software in order to perform statistical analysis and visualize maximal aggregation values in form of bar charts. Optionally, each individual point of the aggregation curves can be exported successively into a spreadsheet software and then to statistical software (*e.g.* GraphPad) in order to visualize the curves. Some investigators use the maximal slope of the aggregation curve to calculate the velocity and the area under the curve to assess platelet activity. The lag time and shape change can also be visualized graphically.

A typical example of an aggregation curve of washed human platelets under control conditions (H_2O) is shown in **Figure 2A**. Addition of the agonist (collagen), induced (after a brief delay) a depression in the aggregation curve caused by the shape change of the platelets. Then, the percentage of aggregation gradually increased over time until a maximum value was reached at about 3-4 min. A slight decrease in the percentage of aggregation was observed towards the end of the 6 min recording, which reflects some disaggregation. As illustrated in **Figure 2A-B**, preincubating human washed platelets with the Panx1 channel inhibitor Brilliant Blue FCF, at a concentration of 1 mM, slowed down or totally abolished the initial platelet shape change and blocked collagen-induced aggregation of washed platelets obtained from 5 different unrelated healthy volunteers. When platelets were preincubated with a lower concentration (100 μM) of Brilliant Blue FCF, the inhibitory effect of the dye on collagen-induced responses and shape change were not observed anymore (see **Figure 2A** for an example). Quantification of the platelet aggregation responses induced by 1 $\mu g/mL$ collagen revealed that 1 mM Brilliant Blue FCF significantly reduced maximal aggregation responses as compared to control conditions as well as to a lower concentration (100 μM) of Panx1 inhibitor (**Figure 2C**). Inhibition of platelet aggregation was specific for collagen-induced responses and not due to undesired side-effects of Brilliant Blue FCF (on cell viability, for example) as the same concentration of the dye (1 mM) did not affect the aggregation response induced by another agonist, *i.e.* 75 μM AA, as illustrated in **Figure 2D**. These results confirm the specific role of Panx1 channels in collagen-induced aggregation responses of human platelets that we and others have previously shown with other pharmacological inhibitors of Panx1 such as probenecid, mefloquine, and the specific ^{10}P anx1 peptides^{7,8}.

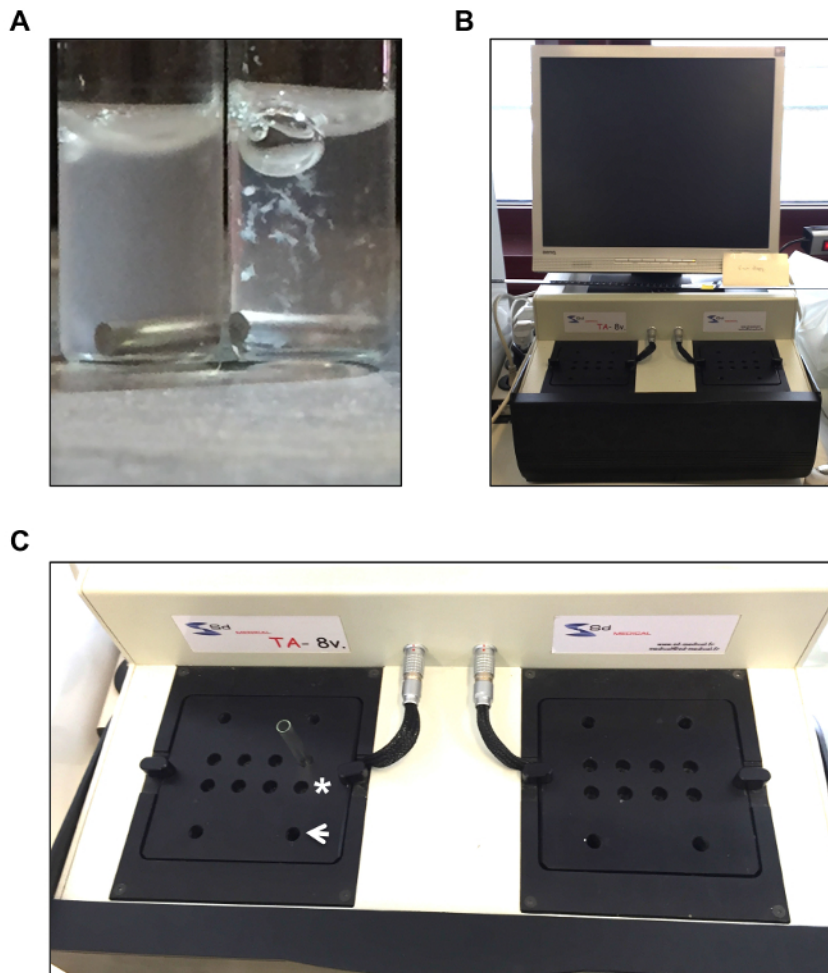


Figure 1: Aggregometry. (A) Representative image of glass cuvettes (containing a stirring magnet) used for aggregometry. The cuvette on the left shows resting platelets while the cuvette on the right illustrates platelet aggregates after collagen-induced activation. (B) Representative image of an 8-well aggregometer for turbidimetric measurements. The wells used (asterisk) to incubate platelet suspensions present in a glass cuvette at 37 °C, and those used for the measurements (white arrow) are indicated in C. [Please click here to view a larger version of this figure.](#)

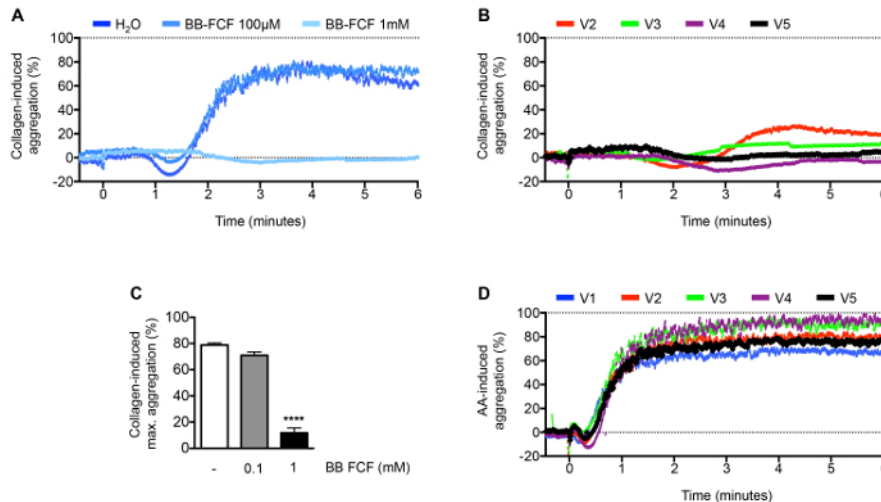


Figure 2: A high Concentration of Brilliant Blue FCF Blocks Platelet Activation in Response to Collagen. (A) Representative traces of collagen-induced aggregation of human washed platelets obtained from the same healthy volunteer (V1) under control conditions (H₂O; dark blue) or after 7 min preincubation with the Panx1 inhibitor Brilliant Blue FCF at 1 mM (light blue) or at 100 μM (intermediate blue color). Aggregation traces were recorded for 6 min. (B) Representative traces of collagen-induced aggregation of human washed platelets obtained from 4 healthy volunteers (V2-V5) after 7 min preincubation with the Panx1 inhibitor Brilliant Blue FCF (1 mM). (C) Quantification of maximal aggregation responses of human washed platelets under control conditions (white bar) or after 7 min preincubation with Brilliant Blue FCF at 100 μM (grey bar) or at 1 mM (black bar). N = 5. ****P < 0.0001; ANOVA followed by Bonferroni's post-test for multiple comparison; Results are expressed as mean ± SEM. (D) Representative traces of AA-induced aggregation of human washed platelets obtained from 5 healthy volunteers (V1-V5) after 7 min preincubation with the Panx1 inhibitor Brilliant Blue FCF (1 mM). [Please click here to view a larger version of this figure.](#)

Discussion

There is great interest in finding new drugs capable of modulating platelet function in order to prevent thrombosis without enhancing the risk of bleeding. For this purpose, *in vitro* laboratory tests which can reliably and reproducibly monitor aggregation responses in human platelets are absolutely necessary. Turbidimetric aggregometry is an easy technique to perform. However, some precautions need to be kept in mind. The measurements need to be performed under continuous stirring as the aggregation process is largely dependent on stirring. It is also important to keep the platelets at a physiological temperature of 37 °C in order to avoid any type of premature activation. Preactivation of platelets can also occur during blood collection and washed platelets preparation, leading to spontaneous aggregation. Thus careful measures should be taken during the complete procedure of platelet preparation.

Turbidimetry is based on the measurement of the OD of the platelet suspension. This makes the technique unsuitable for aggregation measurement in whole blood due to the presence of red blood cells. Thus, turbidimetry can be performed only on PRP or washed platelets isolated from PRP. Although easy to deal with and rather inexpensive, turbidimetry has been recognized as insensitive to the presence of microaggregates^{14,15}. When microaggregates are expected to be of critical importance, impedance aggregometry, which measures the variation in electrical resistance when platelets adhere to an electrode immersed in a citrated whole blood sample, is better suited¹⁶. The platelet aggregation process is also largely dependent on platelet count in the platelet suspension¹⁷; flow cytometry is used in patients suffering from thrombocytopenia¹⁸, from whom the maximum number of platelets that can be obtained is insufficient for turbidimetry. However, the general advantage of turbidimetry is that it allows a detailed analysis of platelet reactivity, such as shape change and disaggregation, which cannot be assessed in whole blood samples.

It is important to realize that natural variation in human platelet aggregation exists due to differences in age, sex and life style as well as health status of the subject whose platelet function is challenged^{19,20,21}. Such natural variation may be on the order of 10-20% in the turbidimetric outcomes, thus care should be taken, when performing and interpreting these experiments, in obtaining platelets from sufficient numbers of unrelated healthy volunteers to allow reliable statistics. This limitation is however counterbalanced by the fact that turbidimetric measurements are highly reproducible, at least when aggregation procedures are well standardized across laboratories. Standardization efforts have been made by the International Society of Thrombosis and Haemostasis²². For this reason, turbidimetry is still the most commonly encountered test for the measurement of platelet aggregation in both clinical and basic science laboratories and remains a technique of choice for studying the effect of drugs on platelet responses.

In the present study we have demonstrated, using turbidimetry to measure responses in human washed platelets, that a commonly-used food dye Brilliant Blue FCF affects platelet aggregation. Elegant electrophysiological studies have revealed a specific blockade of Panx1 channels by this dye^{9,23}. Indeed, high concentrations (1 mM) of this dye showed inhibitory effects on both collagen-induced shape change and maximal aggregation but 10-fold lower concentrations (100 μM) of the dye did not affect the platelet aggregation response. According to the Scientific Opinion on the re-evaluation of Brilliant Blue FCF (E133) as a food additive, published by the European Food Safety Authority²⁴, the maximal allowable concentration of Brilliant Blue FCF (molecular weight = 792.84 g/mol) is 500 mg/kg of food. For H₂O, this would correspond to 0.63 mM, which is a 1.59-fold lower concentration than the one inhibiting collagen-induced platelet aggregation in our experiments. Assuming that only a small fraction of the dye will be absorbed in the intestines after oral ingestion, and that the dye will finally be diluted in the 5-L volume of blood of an adult person, the daily intake of this blue food dye likely has to largely exceed the normal levels before any side-effects on platelet aggregation may be anticipated. Possible side-effects of the highest concentration of the dye in our experiments on platelet viability, for example,

were excluded by the presence of solid aggregation responses to another agonist (AA). These unaffected AA responses also confirmed that inhibitory effects of Brilliant Blue FCF seem to specifically involve the collagen signalling pathway. This is in-line with our earlier study⁷ detailing the specific place for ATP release through Panx1 channels in the collagen-induced platelet aggregation response.

By adapting the protocol of washed platelet isolation to this study, we describe a simple and straightforward method for isolating platelets from human blood. After several washing steps performed essentially by centrifugation, platelets are resuspended in a medium that respects precise physiological conditions including pH, working temperature and concentration of divalent ions but assures the absence of plasma proteins. This is an advantage of this technique over alternative methods such as gel filtration in which elimination of plasma components does not occur²⁵.

All the steps of the washing procedure are of crucial importance to obtain reproducible results when using washed platelets. A plethora of drugs may influence platelet reactivity, thus it is essential that the healthy volunteers are asked to not take any medication for at least 10 days preceding blood collection. In addition, blood collection necessitates that attention is paid to avoid vein trauma or too slow flow as this may cause the generation of thrombin and subsequent platelet activation²². Along the same lines, potential platelet activation during the centrifugation and washing steps can be avoided by the repeated use of PGI₂. Due to its very low stability, PGI₂ should be added to each washing step immediately before each centrifugation. The resuspension of platelets in TA buffer (at a physiological pH) containing apyrase ensures that platelet responses remain intact. The presence of apyrase in the platelet suspension is essential to permit the degradation of ATP and ADP in order to preserve platelets from a desensitization of their purinergic receptors. As the Panx1 signaling pathway involves P2X1 receptor activation in human platelets upon collagen receptor activation, we added a relatively high concentration of apyrase (0.32 U/mL) to the platelet suspension in order to avoid P2X1 desensitization⁷. To avoid desensitization of other purinergic receptors in platelets, lower concentrations of apyrase would be sufficient¹⁰.

Although the procedure of serial centrifugations and washings steps are labor intensive and require more time, the washed platelets obtained using this protocol offer the benefit of higher stability at 37 °C (5-8 h) as compared to platelets used directly from PRP (1-3 h). However, the choice of using PRP or washed platelets should be made cautiously and should include considerations such as the quantity of blood available for the experiment, the agonist to be used as well as the question that will be addressed. For example, as binding of fibrinogen to activated $\alpha 2\beta 3$ integrins is the main mechanism mediating platelet aggregation, absence of fibrinogen in washed platelets might influence the reaction; in particular, when weak agonists (such as ADP) are used¹³. This problem is less critical when a powerful agonist is used, such as thrombin or collagen, which are by themselves able to induce the release of fibrinogen from α -granules. Still, we routinely add exogenous fibrinogen to the washed human platelet suspension in order to increase the amplitude of the aggregation response induced by collagen.

In conclusion, by using human washed platelets and turbidimetry, we found that the food dye Brilliant Blue FCF, through its inhibitory effects on Panx1, represents a potential inhibitor of platelet function.

Disclosures

The authors have nothing to disclose.

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