Regular Article

Preanalytical treatment of EDTA-anticoagulated blood to ensure stabilization of the mean platelet volume and component measured with the ADVIA counters

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Introduction

The Advia® 2120 Hematology System (Siemens Medical Solutions Diagnostics) is an automated analyzer that in addition to measuring the conventional hematologic indices, also provides some information about platelet activation [1]. This system measures the intensity of light scattered by platelets at two different angles (2° and 15°) and from the paired values compute the platelet volume (PV) and the platelet component (PC) concentration on a cell by cell basis. These values are then averaged to provide the Mean Platelet Component (MPC) expressed in g/dL.

The MPC parameter measured by the new generation of ADVIA blood cell analyzers provides direct information on density, or granularity, of platelets and could become a useful biomarker to detect in vivo platelet activation. Unfortunately, it is largely affected by time and storage conditions in standard anticoagulants based on EDTA. The present study was designed to improve the stability of the MPC in blood specimens to facilitate a more standardized use in different laboratories. Blood from healthy controls was collected into EDTA plus additives, and stored at different conditions. MPC and the mean platelet volume (MPV) were assessed at 30 min and at 1, 3, 6 and 24 hours after blood drawing on the ADVIA 2120 system. Flow cytometry was used to evaluate platelet-activation proteins. Ultrastructural morphology of platelets was assessed using electron microscopy. Storage in EDTA increased MPV, decreased MPC, reduced the number of α-granules, and induced changes in the phosphorylation patterns of platelet proteins. A solution based on EDTA containing wortmannin and tyrphostin (ED-WORTY), both inhibitors of signaling pathways, provided good stability for most of the parameters tested up to 6 hours at room temperature. Storage at lower temperatures produced more favorable results. ED-WORTY solutions preserved adequate morphology and had minimal influence on other parameters provided by the ADVIA 2120 system. Thus, the additives included in ED-WORTY may be useful for maintaining the stability of MPC for prolonged periods and to facilitate the transport and exchange of samples among institutions and laboratories.

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Abstract

The mean platelet component (MPC) parameter calculated by the ADVIA blood cell analyzers provides direct information on density, or granularity, of platelets and could become a useful biomarker to detect in vivo platelet activation. Unfortunately, it is largely affected by time and storage conditions in standard anticoagulants based on EDTA. The present study was designed to improve the stability of the MPC in blood specimens to facilitate a more standardized use in different laboratories. Blood from healthy controls was collected into EDTA plus additives, and stored at different conditions. MPC and the mean platelet volume (MPV) were assessed at 30 min and at 1, 3, 6 and 24 hours after blood drawing on the ADVIA 2120 system. Flow cytometry was used to evaluate platelet-activation proteins. Ultrastructural morphology of platelets was assessed using electron microscopy. Storage in EDTA increased MPV, decreased MPC, reduced the number of α-granules, and induced changes in the phosphorylation patterns of platelet proteins. A solution based on EDTA containing wortmannin and tyrphostin (ED-WORTY), both inhibitors of signaling pathways, provided good stability for most of the parameters tested up to 6 hours at room temperature. Storage at lower temperatures produced more favorable results. ED-WORTY solutions preserved adequate morphology and had minimal influence on other parameters provided by the ADVIA 2120 system. Thus, the additives included in ED-WORTY may be useful for maintaining the stability of MPC for prolonged periods and to facilitate the transport and exchange of samples among institutions and laboratories.

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storage conditions, being less reliable after 3 h of sample collection when samples are stored in standard EDTA tubes [1,13]. To prevent this limitation, it is recommended to maintain blood into a mixture of citrate, theophylline, adenosine, and dipryridamole (CTAD), stored at 4 °C, and analyzed between 60 and 180 min after extraction for an optimal preservation of MPC values on the Advia 120®[13].

Despite this recommendation, blood collection and storage conditions could still be sufficiently improved to guarantee more standardized conditions for use in different laboratories, and to allow the analytical measurements to be performed even later than 3 h after blood extraction. In the present studies we have investigated inhibitory strategies that may prolong the stability of the MPC in normal human blood samples anticoagulated and stored in current EDTA tubes based on the deleterious effect of EDTA on platelets. The effect of two inhibitors of signaling pathways, wortmannin and tyrphostin, were investigated.

Material and Methods

General design

Whole-blood was drawn through a 21 gauge needle and immediately transferred to Vacutainers containing standard EDTA (BD Vacutainer), or an alternative anticoagulant combined with different additives. Samples were stored at room temperature (RT) or at 4 °C and analyzed immediately at 30 min, 1, 3, 6 and 24 hours after venipuncture. In each sample, we measure platelet counts, the MPC, and the mean platelet volume (MPV), using ADVIA 2120 Cell Analyzer. Flow cytometry was used for detection of platelet activation markers (CD62P).

Ultrastructural studies were performed in platelets using platelet rich plasma (PRP). In order to analyze the effect of EDTA and the additives included in the solution on platelet activation, tyrosine phosphorylation of proteins was evaluated by SDS-polyacrylamide electrophoresis and western-blotting.

Informed consent was obtained from all donors (n = 12) for blood utilization. The study was approved by the Ethical Committee of the Hospital Clinic, and was carried out according to the principles of the Declaration of Helsinki.

Inhibitory and stabilizing strategies

Blood samples were anticoagulated with either EDTA or an alternative solution under study as stabilizing strategy. ED-WORTY solutions contained 4.64 mM tripotassium EDTA, 1.30 microM wortmannin, 50.45 microM tyrphostin 47, 1.111 mM trisodium citrate 2H2O, 0.177 mM citric acid H2O, 0.2 mM sodium hydrogen phosphate and, 0.75 mM dextrose (all final concentrations). Samples in both solutions were kept at either room temperature or at 4 °C to assess the effect of temperature.

Wortmannin is a fungal metabolite that specifically inhibits phosphatidylinositol 3-kinase (PI3), mitogen-activated protein kinase (MAPK), and myosin light chain kinase (MLCK) [14]. Tyrphostin 47 is one of a series of small molecular weight inhibitors of epidermal growth factor (EGF) receptor kinase activity which were designed to bind to the substrate subsite of the protein tyrosine kinase (PTK) domain [15].

Determination of Hematological Parameters with the ADVIA 2120

Hematological parameters, and more specifically platelet counts, MPV, and MPC were determined using the ADVIA 2120 hematology system (Siemens Healthcare, Barcelona, Spain). Platelet counts in blood samples anticoagulated with some additives were corrected for dilution (dilution factors were considered for recalculations). Blood film extensions were prepared from each sample, fixed and stained with May Grünwald-Giemsa.

ADVIA units were set up in accordance with the manufacturer’s recommendations. Systems were calibrated and standardized before use with ADVIA SETpoint Hematology Control. The ADVIA 2120 Platelet Density Channel standardization process was regularly performed and updated when necessary at a web address (https://secure.cred.ca/ba120/cgi/login.asp?flash=yes) provided by the manufacturer. This standardization step was considered as a starting point of reference for all subsequent experimental research, or developmental analysis on the ADVIA 2120.

Flow Cytometry to detect modifications in CD62P

Monoclonal antibodies (MoAbs) were used for specific labelling of membrane glycoproteins. MoAbs used were commercially available (Immunotech, Marseille, France) and were purchased conveniently tagged with fluorescein (FITC) or phycoerythrin (PE). GPIIb-IIIa was labeled with anti CD41a-PerCP and P-selectin was detected with antibodies to CD62P-FITC. Nonspecific membrane immunofluorescence was determined by using Ig-FITC and Ig-PE.

Flow cytometry analysis was performed on whole blood samples as previously described [16,17]. Samples were analyzed in a FACScan flow cytometer using Lysys II 1.1 program (Becton-Dickinson, Mountain View, CA). Platelets were identified by gating Per-CP labeling corresponding to the MoAb to GPIIb-IIIa antigens and by their characteristic light scatter. Binding of different antibodies was determined by measuring the corresponding FITC fluorescence on 5000 individual platelets. The flow cytometer was calibrated with

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Table 1

<table>
<thead>
<tr>
<th>PTls</th>
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<th>ED-WORTY</th>
<th>ED-WORTY 4 °C</th>
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<td>265.2 ± 20.7</td>
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<td>264.3 ± 22.6</td>
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<tr>
<td>3 h</td>
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<td>257.7 ± 21.8</td>
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<td>6 h</td>
<td>261.4 ± 21.6</td>
<td>254.8 ± 23.3</td>
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<td>24 h</td>
<td>261.4 ± 21.6</td>
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Cell counts in blood samples at the time of extraction (0) and after 30 min, 1 h, 3 h, 6 h, and 24 h of preservation in EDTA, ED-WORTY, and ED-WORTY at 4 °C. No changes were observed in platelet (PLT), red blood cells (RBC), and white blood cells (WBC) counts at any of the conditions evaluated. Results are expressed as Mean ± SEM (n = 12).
2 μm Calibre beads (Becton-Dickinson). Binding for MoAb to GPIIb-IIIa was expressed as percentage of mean fluorescence intensity (MFI) over baseline values. For the study of CD62, a threshold was set at baseline level in order to delimit a 2% of platelet subpopulation with the highest immunofluorescence. This 2% was used as a threshold to determine the proportion of platelets which exceeded those levels in subsequent samples.

Ultrasturctural studies

Sections

Samples of platelet rich plasma (PRP) were obtained from blood samples preserved either with EDTA or with ED-WORTY at RT and at 4 °C, at baseline and 6 h and 24 h after extraction. PRP samples were obtained by centrifugation at 200×g for 10 min, at 22 °C, and combined with an equal volume of 2.5% glutaraldehyde in White's saline [18] for 5 min, then centrifuged. The supernatant was removed and replaced with 3% glutaraldehyde in the same buffer. The suspensions were maintained at 4 °C for 30 min, then centrifuged. Pellets were suspended in a solution of 1% osmium tetroxide in distilled water containing 15 mg/ml potassium ferrocyanide (pH 7.4) for 90 min at 2 °C. Glutaraldehyde-tannic acid fixatives were also applied to emphasize the open canalicular system [19]. After osmification, samples were dehydrated in a graded series of ethanol concentrations, then treated with propylene oxide and embedded in Epon 812. The sections were stained with uranyl acetate and lead citrate to enhance contrast. Examination and alpha granules count were carried out in a Phillips (Mahwah, NJ, USA) 301 electron microscope.

Whole mount electron microscopy

Platelet dense bodies count was performed by whole mount electron microscopy. PRP samples were placed on copper grids and treated as described elsewhere [20]. The grids were air dried and examined using a Philips model 301 electron microscope.

Analysis of tyrosine phosphorylated proteins in platelets

In order to analyze the effect of EDTA and the additives included in the ED-WORTY solution on platelet activation, tyrosine phosphorylation of proteins was evaluated by SDS-polyacrylamide gel electrophoresis and western blotting. Blood was obtained from healthy donors and collected into citrate/phosphate/dextrose (CPD) at a concentration of citrate of 19 mM. Platelets were separated as platelet resuspended in Hanks balanced salt solution with Ca2+, and kept under resting conditions for 30 min at 37 °C. Finally, the samples were lysed by addition of Laemmli's buffer (125 mM Tris-HCl, 2% SDS, 5% glycerol and 0.003% bromophenol blue) containing 2 mM orthovanadate and 0.625 mg/ml N-ethylmaleimide, for 15 minutes. Analysis of tyrosine-phosphorylated proteins was performed on proteins previously resolved by gel electrophoresis, as described elsewhere [22].

Statistics

Results were expressed as mean±standard error of the mean (S.E.M.). Statistical analysis was performed by means of a paired twotailed Student’s t test and a p<0.05 was considered statistically significant.

Results

Evolution of MPC and MPV during storage

Values of MPC for platelets exposed to standard EDTA anticoagulant averaged 24.9±6.1 g/dL (mean±SEM, n = 12) immediately after blood drawing (time 0). As shown in Fig. 1, MPC increased slightly after 30 min of storage and values remained basically stable between 30 and 60 min (25.8±5.6 g/dL and 25.2±5.3 g/dL). After this period, MPC tended to progressively decrease reaching values of 23.9±5.7 g/dL and 22.9±5.9 g/dL after 3 and 6 hours, respectively. Reductions in MPC revealed statistical differences (p<0.05 and p<0.001, respectively, vs baseline values) in blood samples exposed for 3 or more hours to EDTA at room temperature. Values for the MPC at 24 hours were markedly decreased, reaching levels of 20.1±4.3 g/dL (p<0.001 vs. baseline values).

In contrast with the decreasing tendency of MPC, MPV tended to progressively increase with the storage in EDTA (Fig. 1). Values of MPV for platelets exposed to standard EDTA anticoagulant averaged 8.35±0.27 fl (mean±SEM, n = 12) immediately after blood drawing. MPV values tended to progressively increase to values of 8.64±0.29, 9.22±0.35, and 9.52±0.31 fl, at 1, 3 and 6 hours, respectively. The increase in MPV reached levels of statistical differences (p<0.05 vs baseline values) in blood samples exposed for 6 or more hours to EDTA. Values for the MPV at 24 hours were markedly increased reaching levels of 10.63±0.35 fl.

The presence of inhibitors (ED-WORTY) in the EDTA anticoagulated tubes clearly improved the stability of the MPC. Values for this parameter remained almost indistinguishable from baseline values during the 6 initial hours of storage at room temperature. A statistically significant decrease was observed in measurements performed in samples of blood stored for 24 hours. The stability in the MPC parameter observed with ED-WORTY was more evident when samples were stored at 4 °C and paralleled the similar level of preservation in MPV (see Fig. 1). No significant changes were detected for blood cell counts (Table 1).

Expression of the activation dependent antigen CD62P on the platelet surface

Expression of CD62P at the platelet surface was evaluated by flow cytometry in samples stored for 30 min, 6 h, and 24 h in the presence of EDTA and WORTY (Fig. 2). In the presence of EDTA, expression of
CD62P was minimally detected after 30 min of storage (% of positive platelets of 2.8 ± 0.5, Mean ± SEM, n = 6) and increased significantly to 14.5 ± 2.8% and >40 % after 6 h and 24 h, respectively.

When samples were stored in the ED-WORTY solution, CD62P expression at the platelet membrane was also observed but reaching significantly lower levels. At room temperature, % of positive platelets were of 2.0 ± 0.3, 4.5 ± 1.3 (p < 0.01 vs. EDTA at 6 h), and 31.5 ± 3.8 after 30 min, 6 h, and 24 h, respectively. At 4 °C, % of platelets expressing CD62P in the presence of ED-WORTY were of 1.5 ± 0.3, 6.2 ± 1.8 (p < 0.05 vs. EDTA at 6 h), and 30.8 ± 2.9 after 30 min, 6 h, and 24 h, respectively.

Measurements of intraplatelet granules by electron microscopy

The stability of the sample to allow measurements of MPC in the presence of ED-WORTY was also assessed by counting intraplatelet dense bodies and alpha-granules by electron microscopy (Figs. 3 and 4).

In the presence of EDTA alone, the number of dense bodies decreased progressively from 4.7 ± 0.2 (Mean ± SEM, n = 4 different experiments, baseline value) to 4.1 ± 0.3 and 2.6 ± 0.2 when samples were kept undisturbed for up to 6 h and 24 h, respectively. This effect was not noticed when ED-WORTY was present, since the number of dense bodies was maintained as in the initial sample along the time of storage (5.8 ± 0.3, 4.8 ± 2.8, and 5.3 ± 2.8, at 0 h, 6 h, and 24 h, respectively). Storage at lower temperatures seemed to produce more favorable results (5.9 ± 0.5, 5.3 ± 0.3, and 5.3 ± 0.8, at 0 h, 6 h, and 24 h, respectively).

The number of alpha granules per platelet section in the presence of EDTA decreased from 12.5 ± 4.0 at baseline to 6.7 ± 2.0 (p < 0.05), and 3.5 ± 1.5 (p < 0.05) after 6 and 24 h, respectively. ED-WORTY prevented alpha granule degranulation observed in samples stored in EDTA, with numbers of α-granules per platelet section varying from 12.5 ± 4.3 to 10.7 ± 3.5, and to 10.9 ± 4.7 at baseline, 6 and 24 h respectively. As occurred with dense bodies, storage at lower temperatures seemed to produce more favorable results on numbers for alpha granules per platelet section (12.5 ± 4.3 to 12.0 ± 2.5, and to 11.8 ± 2.7, at baseline, 6 and 24 h, respectively).

Analysis of Tyrosine Phosphorylation

Incubation of washed platelets with EDTA for 20 min caused a mild though general increase in the protein phosphorylation pattern with respect to control platelet samples (see Fig. 5). Proteins p125, p80, p72, and p60 became phosphorylated in the presence of the calcium chelator. The impact of EDTA on protein phosphorylation was more evident on protein p62, which appeared clearly dephosphorylated.

Presence of the ED-WORTY solution inhibited some of the modifications in the phosphorylation patterns induced by exposure to EDTA, although p62 remained dephosphorylated (see Fig. 5).

Discussion

Results from the present study show that storage of blood samples in current anticoagulants based on EDTA resulted in a progressive increase in the MPV, and a decrease in the MPC, with a significant reduction in the number of platelet α-granules as revealed by EM. Exposure to EDTA induced changes in the phosphorylation patterns of platelet proteins. From these observations it can be derived that EDTA causes alterations in platelets that resemble those occurring during platelet activation. Addition of certain inhibitors of protein phosphorylation provided stability for the MPV and for the MPC parameters, and significantly prevented the degranulation caused by EDTA. These additives could be useful to guarantee the stability of MPC and MPV for prolonged periods and to facilitate the transport and exchange of samples among institutions and laboratories.

MPC is a new platelet parameter detected by modern ADVIA cell blood count analyzers that measure the mean refractive index of the platelets. MPC is linearly related to platelet density and is reduced when platelets degranulate, thus indicating that platelets have undergone activation. Several studies have recently demonstrated that MPC values are inversely correlated with platelet membrane P-selectin expression [4,13], and this marker has been used as an indicator of platelet activation in different diseases and physiologic states [23–26]. Thus, measurement of the MPC is becoming a quick
method for the easy detection of platelet activation. In this regard, several lines of evidence seem to confirm the potential use of the MPC parameter, generated on an automated hematology system, to define high risk patients and to monitor the variability of the platelet response to anti-platelet therapies [8,26].

Despite the potential diagnostic value of the MPC parameter, the stability of the blood samples should be improved in order to apply MPC measurements accurately. Current anticoagulants for cell blood counting are based on EDTA. From our present studies it can be derived that EDTA causes a deleterious effect on platelets, as already reported by White et al. [27,28]. From those studies, exposure of platelets to EDTA for prolonged periods results in extreme distortion of their morphology including dilation of the open canalicular system, a progressive tendency to shape change, and even formation of platelet agglutinates. All these external transformations are compatible with activation [27]. Our present results after measuring MPC when samples were stored in EDTA for longer than 2 hours confirm a marked reduction in this parameter, together with decreases in the number of internal granules, and also increases in the expression of CD62P at the platelet surface, as mentioned by others [13]. Interestingly, the presence of the kinase inhibitors wortmannin and tyrphostin, together with citrate, prevented some of the changes observed. Wortmannin is a fungal metabolite that specifically inhibits phosphatidylinositol 3-kinase (PI3K), but also the mitogen-activated protein kinase (MAPK), and the myosin light chain kinase (MLCK) [14], and tyrphostin 47 is a tyrosine kinase inhibitor [15]. Our present results demonstrate that the strategy used is able to prevent the deleterious effect of EDTA on MPC and MPV stability, as confirmed by the maintenance of both parameters along the study, and by the prevention of both the expression of P-selectin and the ultrastructural changes at least for up to 6 hours. These results show a clear advantage with respect to the use of the CTAD anticoagulant reported before [13].

Through the present study, we have observed that exposure of platelets to EDTA for a short period of time promoted tyrosine phosphorylation of certain proteins, events that are related to activation, with dephosphorylation of some constitutively phosphorylated proteins. EDTA is a divalent cation chelator that depletes not only Ca^{2+} but also Mg^{2+} from the media. Our present results would be in agreement with previous findings reporting that EDTA causes activation such as shape change, reassociation and translocation of GPIIb-IIIa [29], and that Mg^{2+} inhibits activation [30–32]. In addition, it is suitable to think that EDTA may also affect receptor-mediated Ca^{2+} entry and, as a consequence, Ca^{2+} mobilization from the internal stores. This process promotes signal transduction. Interestingly, ED-WORTY prevented some of the alterations induced by EDTA indicating that the biochemical mechanisms of these two agents inhibit the deleterious effect of EDTA, and markedly enhance the stability of the MPC. Although tyrphostin 47 may be considered an unspecific agent, tyrosine phosphorylation of proteins plays a key role in platelet activation. In addition, PI3K is involved in the affinity modulation of integrins, in cytoskeletal arrangements, and, interestingly, in calcium signalling [33].

Inhibition

**Fig. 4. Effect of EDTA and ED-WORTY on intraplatelet granules.** Bar diagrams representing the number of alpha-granules per platelet section (upper diagrams) and dense bodies (lower diagrams) in platelets exposed to EDTA and ED-WORTY, at room temperature, for 0, 6, and 24 h. Values are expressed as Mean±SEM, n=4, *p<0.05.

**Fig. 5. Effect of EDTA and ED-WORTY on tyrosine phosphorylation of platelet proteins.** Platelets were exposed to EDTA and ED-WORTY at room temperature for 20 min. Samples were resolved by 8% SDS-PAGE and proteins transferred to nitrocellulose membranes. Phosphotyrosine proteins were detected by a specific antibody and the ECL technique. Images are representative of 6 different experiments.
of PI3K by Wortmannin would prevent calcium mobilization, event that
may be caused by the chelating effect of EDTA.
There are several reasons to use EDTA as anticoagulant: to prevent
blood coagulation, to restrict activation of calcium dependent
proteases, and/or to inhibit platelet contacts. Severe calcium depri-
vation caused by EDTA causes dissociation of GPIIb-IIIa complexes, but
also alterations in the platelet OCS that completely isolate more
internal segments of the OCS from the external milieu [34,35].
Calcium chelation by EDTA has been used to investigate those
mechanisms occurring independently of GPIIb-IIIa in platelets.
Under these conditions, as we have already reported [36], several
experimental inconveniences may occur such as discrepancies in the
distribution of surface glycoproteins, in the recognition of surface
antigens by specific antibodies, and in the agglutination of platelets
with bovine VWF [37]. Therefore, the present and previous observa-
tions make EDTA not suitable as anticoagulant for the research on
platelet function.

In summary, the MPC appears as a very interesting parameter that
offers the possibility to simply and early detect platelet activation under
clinical conditions. Modifications in the levels of MPC may be useful to
detect acute phase platelet activation and during the follow up of
patients with atherothrombosis / cardiovascular events. The main
inconvenience is the maintenance of the stability of the sample in
patients with atherothrombosis / cardiovascular events. The main
detect acute phase platelet activation and during the follow up of

References
mean platelet component to measure platelet activation on the ADVIA 120
2. Brunnritt DR, Barker HF. The determination of a reference range for new platelet
parameters produced by the Bayer ADVIA120 full blood count analyser. Clin Lab
Platelet volume and parameters determined by the Bayer ADVIA 120 in reference
Assessment of platelet activation in several different anticoagulants by the Advia
120 Hematology System, fluorescence flow cytometry, and electron microscopy.
rapid, automated flow cytometric method to measure activated degranulated
Assessment of in vitro platelet activation by Advia 120 platelet parameters. Lab
8. Ahnadi CE, Boughrassa FF, Chapman-Montgomery ES, Poisson V, Gervais A,
Okrongly D, et al. Comparison of two methods to assess variability of platelet
response to anti-platelet therapies in patients with acute coronary syndrome
9. White JG. EDTA-induced changes in platelet structure and function: clot
10. White JG, Escobar G. EDTA-induced changes in platelet structure and function:
11. White JG. Kruimwiede MD, Escobar G. EDTA induced changes in platelet structure
12. White JG. Effects of ethylenediamine tetraacetic acid (EDTA) on platelet structure.
of the anticoagulants EDTA and citrate, theophylline, adenosine, and dipyrindamide
(CTAD) for assessing platelet activation on the ADVIA 120 hematology system.
histamine secretion by wortmannin through the blockage of phosphatidylinositol
the addition of second-messenger effectors to platelet concentrates separated from
collected whole blood donations and stored at 4°C or –80°C. Transfusion 2000;40:227–34.
Glycoproteins on platelet membrane in inherit macrothrombocytopenias. Thromb
19. Escobar G, Leistikow E, White JG. The fate of the open canalicular system in surface
et al. Evidence for locus heterogeneity in Puerto Ricans with Hermansky-Pudlak
induces platelet aggregation: a morphological demonstration. Br J Haematol
tyrosine phosphorylation of proteins after activation of platelets with thrombin
23. Boos CJ, Bevers GD, Lip GY. Assessment of platelet activation indices using the
ADVIATM 120 amongst ‘high-risk’ patients with hypertension. Am Med J 2007;39:
72–8.
24. Chung I, Choudhury A, Lip GY. Platelet activation in acute, decompensated congesitive
25. Chung I, Choudhury A, Patel J, Lip GY. Soluble CD40L. platelet surface CD40L and
total platelet CD40L in congestive heart failure: relationship to platelet volume,
component as an indicator of platelet activation in foals and adult horses. J Vet
27. White JG. Effects of ethylenediamine tetracetic acid (EDTA) on platelet structure.
28. White JG. Kruimwiede MD, Escobar G. EDTA induced changes in platelet structure
29. Ma Y, Wong K. Role of extracellular magnesium in platelet activation and spread-
30. Fitch FW, Fitch TF. Prolonged platelet function and survival in platelet transfu-
31. Fitch FW, Fitch TF. Prolonged platelet function and survival in platelet transfu-
32. Fitch FW, Fitch TF. Prolonged platelet function and survival in platelet transfu-
33. Fitch FW, Fitch TF. Prolonged platelet function and survival in platelet transfu-
34. Fitch FW, Fitch TF. Prolonged platelet function and survival in platelet transfu-
35. Fitch FW, Fitch TF. Prolonged platelet function and survival in platelet transfu-
36. Fitch FW, Fitch TF. Prolonged platelet function and survival in platelet transfu-
37. Fitch FW, Fitch TF. Prolonged platelet function and survival in platelet transfu-