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Critical Reviews in Oncology/Hematology xxx (2014) xxx–xxx

Hematology
Incorporating Geriatric Oncologywww.elsevier.com/locate/critrevonc

Biologicals, platelet apoptosis and human diseases: An outlook

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Accepted 10 November 2014

Contents

1. Introduction	00
2. Physiological relevance of platelets and platelet-derived microparticle generation	00
3. Platelet apoptosis and microparticles – role in pathological conditions	00
3.1. Bleeding disorders	00
3.2. Cardiovascular disorders	00
3.3. Cancer	00
3.4. Malaria	00
3.5. Arthritis	00
3.6. Diabetes	00
3.7. Hyperthermia and dengue	00
3.8. Alzheimer's disease	00
4. Effect of pro-apoptotic biologicals on platelets	00
5. Platelet anti-apoptotic biologicals and their application in auxiliary therapy	00
6. Future directions and conclusion	00
Conflicts of interest	00
Reviewers	00
References	00
Biographies	00

Abstract

Platelets, once considered mediators of hemostasis and thrombosis, are now known to be involved in wound healing, inflammation, cardiovascular diseases, diabetes, arthritis, and cancer. Recent reports attest that platelets possess the cellular machinery to undergo apoptosis and that platelet apoptosis can be triggered by myriad stimuli including chemical and physical agonists, and pathophysiological conditions. Augmented rate of platelet apoptosis leads to thrombocytopenia, bleeding disorders and microparticle generation. Despite knowing the significant role of platelets in health and disease, and that any alterations in platelet functions can wreak havoc to the health, the offshoot reactions of therapeutic drugs on platelets and the far-reaching consequences are often neglected. The present review focuses on the impact of

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platelet apoptosis and the role of platelet-derived microparticles on different pathophysiological conditions. It also touches upon the effects of biologicals on platelets, and discusses the need to overcome the adverse effects of pro-apoptotic drugs through auxiliary therapy.
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Keywords: Platelet apoptosis; Microparticles; Biologicals; Cardiovascular diseases; Cancer

1. Introduction

Platelets were once considered mere mediators of hemostasis and thrombosis. However, rigorous research in the field of platelet biology gave a new insight into their dynamic and versatile characteristics. It is amazing that these seemingly simple anuclear cells, which are unique to mammals, have such a vast array of physiological functions [1]. They play crucial roles in pathophysiological conditions including wound healing, inflammation, cardiovascular diseases (CVDs), diabetes, arthritis, Alzheimer's disease (AD), angiogenesis and metastasis. Till two decades ago, it was not known how exactly their numbers is controlled or how they undergo death. Recent reports prove beyond doubt that platelets do undergo programmed cell death via apoptosis. Apart from cellular senescence, platelet apoptosis is also triggered by various stimuli including chemical agonists (e.g., thrombin, collagen, ADP, hydrogen peroxide, arachidonic acid, epinephrine, calcium ionophore-A23187, etc.), oxidative stress-induced pathological conditions (e.g., hyperlipidemia, Kawasaki disease, Bernard–Soulier syndrome, altered cardiac functions, type-2 diabetes and chronic uremia) and physical factors (e.g., hyperthermia, platelet storage under standard banking conditions, shear stress) [2].

Bone marrow megakaryocytes undergo apoptosis *de facto* to release platelets. As such, platelets possess the cellular machinery (which is derived from their parent cells) required for normal functioning. This implies that they also undergo apoptosis like any other nucleated cell, except for the nuclear apoptotic events. However, till date there is strong evidence only for the intrinsic apoptotic pathway and very few studies on the extrinsic pathway in platelets. The former occurs through mitochondrial dysfunction in response to stress. Apoptotic platelets display elevated levels of reactive oxygen species (ROS), calcium (Ca^{2+}), cytochrome c, apoptotic protease-activating factor-1 (Apaf1) and caspases-9 and -3, which are all considered markers of apoptosis. In addition, platelets undergoing apoptosis also express surface markers such as phosphatidylserine (PS) (Fig. 1) [3]. Recent reports suggest the involvement of cell signaling pathways such as, p38 MAPK/cPLA2, PI3K, extracellular signal-regulated protein kinase (ERK) and c-Jun NH2-terminal kinase (JNK) in apoptotic platelets [4–7]. Further, there are various lines of evidence suggesting the existence of extrinsic apoptotic pathway in platelets, but none decisively prove it. For instance, though the presence of TNF- α (a prominent cytokine regulating extrinsic apoptotic pathway) in platelets is controversial,

accumulation and secretion of a range of TNF- α -related ligands like Fas-L, TRAIL, TWEAK and LIGHT, is observed [8]. Stored platelets are also found to express mRNA and proenzyme for caspases-8 and -10, mRNA for death receptors such as, DR3, DR4, DR5, TRAIL, TNF receptor p55, and RIP, as well as elevated concentration of TNF α [9]. Moreover, caspase-8 activation in platelets stimulated with TPEN and resveratrol was also reported [10,11].

When the rate of platelet apoptosis exceeds the normal physiological level, it may have far-reaching consequences: (i) reduced platelet count (thrombocytopenia) leading to bleeding disorders; (ii) generation of microparticles (MPs), which play a major role in the propagation of various pathological conditions such as, CVDs, cancer, type-2 diabetes and arthritis [12]. Platelet-derived MPs (PMPs) constitute around 80% of circulating MPs. A study by Berckmans et al. determined the concentration of MPs of different cellular origin in fresh blood samples via flowcytometry to be $237 \times 10^6/\text{L}$ (platelet-derived), $28 \times 10^6/\text{L}$ (erythrocytes-derived), $46 \times 10^6/\text{L}$ (granulocyte-derived) and $64 \times 10^6/\text{L}$ (endothelial cell-derived) [13]. MPs are vesicle-like structures surrounded by plasma membrane bilayer enclosing enzymes, transcription factors and mRNA [14,15]. Apoptotic platelets shed MPs via dynamic membrane blebbing that is driven by the contractile force of cytoskeletal structures actin–myosin [16]. Activated Rho-associated kinase augments the actin–myosin force generation. The shed MPs contain a surfeit of biomolecules including proteins (signal proteins, receptors, cytoskeleton, and effector proteins), lipids, and nucleic acids. They express several surface antigens such as, GpIb, PECAM-1, GpIIb-IIIa, P-selectin, CD 63, CD41a, and CD 61 [17]. However, the surface protein content may be different from that of the plasma membrane of the parent cell, since the inclusion of protein molecules into MPs can be selective and regulated by the stimuli from specific agonists or microenvironments of the parental cells [18].

The present review mainly focuses on the impact of platelet apoptosis and the role of MPs on different pathological conditions. Owing to the extremely sensitive nature of platelets, they are very much vulnerable to therapeutic drugs in the circulation. The surfacing of recent reports of commonly used therapeutic drugs provoking platelet apoptosis is of serious concern, considering the prominent role played by platelets in health and disease. Thereby, the review also touches upon the effects of therapeutic drugs on platelet apoptosis and MP generation, and their clinical consequences. Finally, the need to overcome the

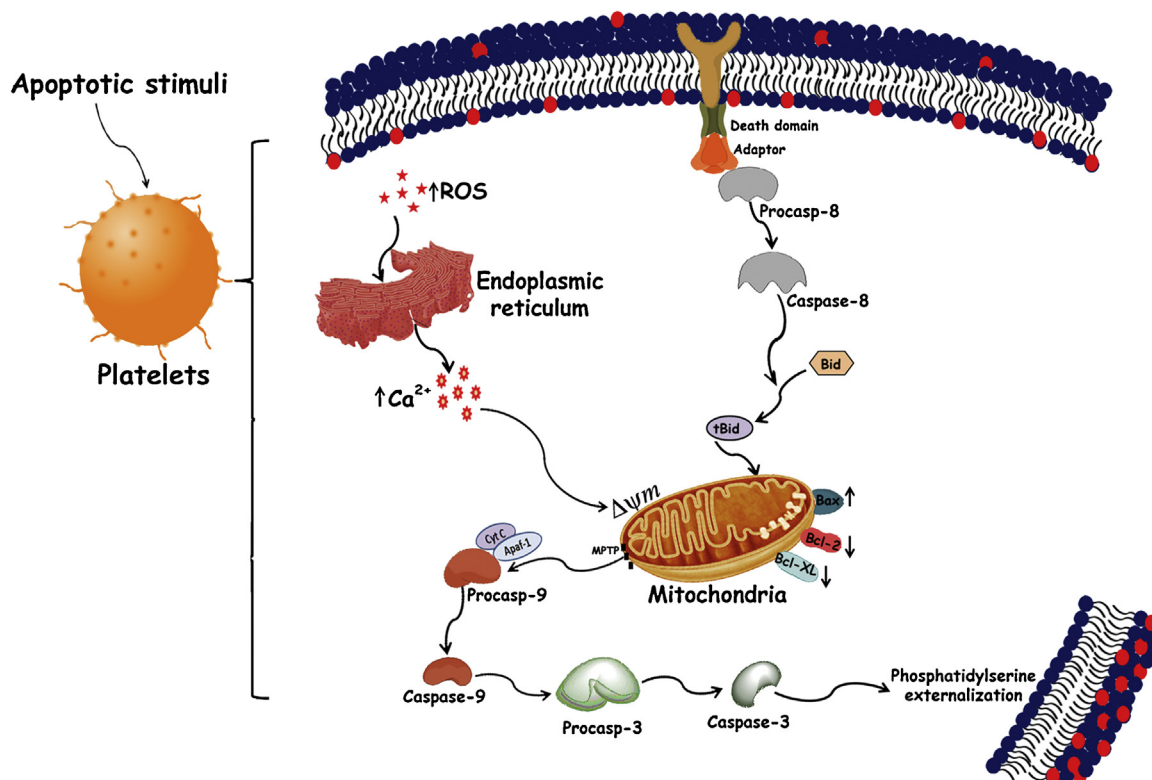


Fig. 1. General mechanism of platelet apoptosis.

adverse effects of pro-apoptotic drugs through auxiliary therapy is stressed upon, wherein the biologicals that display anti-apoptotic effects on platelets are discussed.

2. Physiological relevance of platelets and platelet-derived microparticle generation

Platelets are essentially destined to curb bleeding; nevertheless they have diverse functions beyond hemostasis and thrombosis. They recruit leukocytes and progenitor cells to vascular injury and inflammation sites, secrete pro-inflammatory, anti-inflammatory and angiogenic factors and MPs into the circulation, and also stimulate thrombin generation. Their ability to store and release bioactive molecules makes them key functional modulators of other cells. Platelets also generate lipid-derived mediators such as thromboxane A₂, and participate in transcellular metabolism as well. They are capable of accomplishing extranuclear translation of proteins that include IL-1 β and tissue factor, which connect hemostasis with inflammation [1]. Platelets also have crucial roles in host defense, response to injury, and immune surveillance by acting as circulating sensors that link immune responses to wound healing. Indeed, platelets co-ordinate the whole process of tissue repair by eliciting an inflammatory pathway that involves extracellular matrix remodeling, cell migration, proliferation and differentiation, and angiogenesis [8,19]. They release cytokines, chemokines, and growth

factors including SDF-1 and HGF that mediate recruitment, proliferation and activation of the cells that are involved in wound healing such as, fibroblasts, neutrophils, monocytes, smooth muscle cells and mesenchymal stem cells. Platelets also maintain the balance between cell death and survival by releasing both proapoptotic (Fas-L, CD40L, TRAIL, TWEAK and LIGHT) and antiapoptotic (hepatocyte growth factor, stromal-derived growth factor-1, serotonin, adenosine diphosphate and sphingosine-1-phosphate) factors, which in turn influence the pathophysiology of damaged tissue [8]. Recently, platelets were reported to safeguard the vascular integrity by supporting the semi-permeable barrier function of the resting endothelium [20]. Thus, discrepancies in platelet apoptosis rate might have far reaching consequences than was thought earlier. Drop in platelet count might not only lead to bleeding disorders, but also affect the processes of inflammation, tissue repair, transcellular metabolism, immune surveillance, along with loss of vascular integrity. Thus, the several studies that have reported thrombocytopenia as a secondary complication in a treatment regimen should not be taken lightly as just a condition with low platelet count. Thrombocytopenia might be the beginning of various other health problems caused by the altered biological functions that involve platelets as discussed above. This aspect not only calls for further research but also make amendments in the treatment strategies in drugs that are known to cause thrombocytopenia.

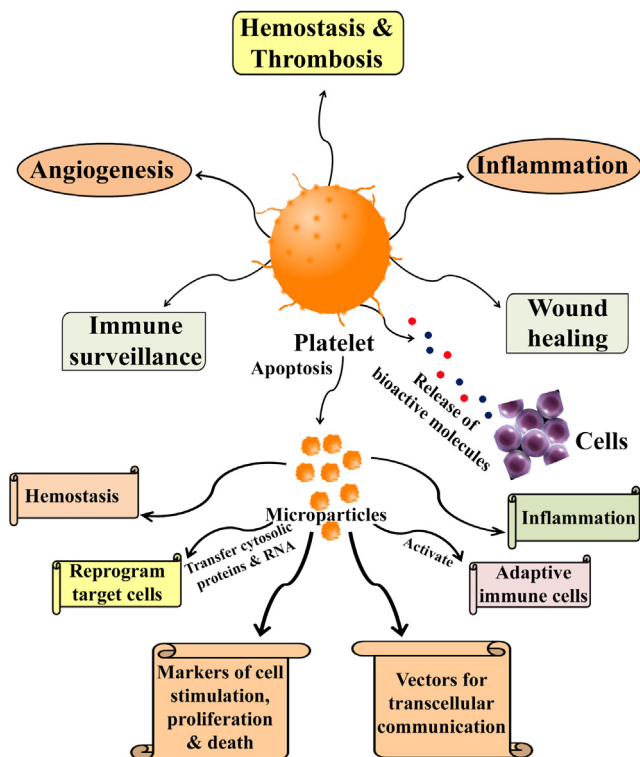


Fig. 2. Role of platelets and platelet derived microparticles in various physiological and pathological events.

Further, PMPs that were long been considered inert ‘platelet dust’ are now reported to wield their effects on other cells as well. Currently, PMPs are deemed as markers that indicate the balance between cell proliferation and death. Because PMPs express procoagulant PS on their surface, a major bulk of them is considered to arbitrate hemostasis under physiologic conditions. Besides their marker functions, MPs have a significant role as vectors in the transcellular communication as they possess various types of receptors and bioactive substances on their surface including cytokines, signal proteins and nucleic acids, in addition to mRNA and miRNA [14,21]. They exchange biological information with target cells either via surface-expressed ligands or by transporting surface receptors from one cell to the other. MPs also have the capacity to reprogram target cells via attaching or fusing and delivering cytoplasmic proteins and RNA to the cells. Further, PMPs have been shown to respond to signals that elicit antibody synthesis and alter lymphocyte activities, and thus activate adaptive immune cells in the particular tissue [22]. PMPs are also involved in tissue repair. They control apoptosis in endothelial and smooth muscle cells, and also provide survival signals to monocytic, endothelial and neural stem cells [8]. Thus, MPs have acquired an exceptional status in human physiology of late, and their role in the maintenance of homeostasis and normal health cannot be ignored (Fig. 2). Hence, it is imperative to monitor the MP levels in patients undergoing treatment with drugs that are known to

cause thrombocytopenia. Unfortunately, the importance of MPs in the healing process and their side-effects are not yet recognized as a serious threat by the medical fraternity. Moreover, simple and effective methods to measure MPs are not yet popularized.

3. Platelet apoptosis and microparticles – role in pathological conditions

Since, platelets and PMPs play such a critical role in normal human physiology, it is quite obvious that disturbances in their numbers and functions might instigate disastrous pathological outcomes (Fig. 3). Platelet response to various disease conditions depends on the surface active markers (e.g., CD36, CD41, CD42a, CD42b, and CD61) and secretory biomolecules [23]. Platelets are known to interfere with the signaling and expression of these molecules in different disease pathologies, some of which are discussed below.

3.1. Bleeding disorders

Thrombocytopenia is a condition wherein the platelet count slumps to 100,000 platelets/ μL of blood, the normal range being 150,000–450,000/ μL of blood. In severe cases the platelet count drops below 10,000/ μL leading to a greater risk of bleeding. Thrombocytopenia seems to be the direct and most obvious consequence of elevated rate of platelet apoptosis. More than 200 drugs reportedly cause immune thrombocytopenia, e.g., antibiotics (rifampin, sulfamethoxazole, vancomycin, sulfonamides, linezolid, etc.), anticonvulsants (carbamazepine, phenytoin, valproic acid), RGD mimetic agents (eptifibatide and tirofiban), anti-inflammatory drugs (acetaminophen, diclofenac, quinine), antineoplastics (interferon- α), cardioprotective drugs (eptifibatide, quinidine, tirofiban), antihypertensive drugs (methyldopa), as well as antidiabetic drugs (chlorpropamide) [12]. However, barring few it is not known whether all these drugs induce platelet apoptosis. Bernard–Soulier syndrome (BSS) is another bleeding disorder, which is characterized by abnormally large platelets that have increased PS surface exposure in the resting state, and reduced rate of survival in the circulation. Activated BSS platelets have been reported to undergo apoptosis-like events, with PS exposure and MP production being enhanced, resulting in their shortened survival, as well as affecting thrombin generation [24].

3.2. Cardiovascular disorders

Kawasaki disease (KD), which is characterized by inflammatory acute febrile vasculitis, is associated with oxidative stress and defective platelet apoptosis. KD is associated with coronary artery damage and other cardiovascular complications. PS-exposing platelets are known to exert a pro-coagulant activity leading to increased risk of vascular complications in KD [25]. In hyperlipidemia (which is

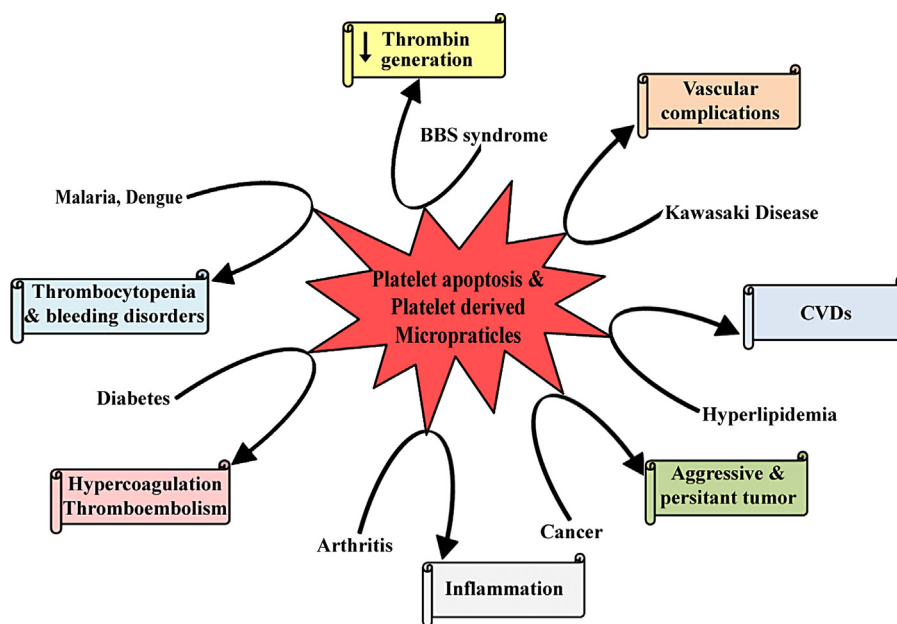


Fig. 3. Different human pathological conditions/disorders responsible for platelet derived microparticle generation and altered platelet functions.

a classical cardiovascular risk factor), some platelets are reported to be in an activated state in circulation, and undergo early apoptosis [26]. Moreover, activated platelets and circulating PMPs possess active surface area with procoagulant aminophospholipids, which support the assembly of the coagulation cascade enzymes [27,28]. Because PMPs express procoagulant PS on their surface, there are possibilities of deviation toward the pathogenesis of arterial thrombotic and thromboembolic complications [22,29]. Acute coronary syndrome is initiated by the erosion and rupture of an atherosclerotic plaque, which disturbs the subendothelial protein matrix allowing platelet-adhesion molecules like vWF factor and collagen to interact with circulating platelets. This leads to platelet activation, shape change and release of storage granules containing platelet agonists such as, ADP and TxA₂, and shape change of platelet fibrinogen receptor GPIIb/IIIa. Besides, already-activated platelets and the released PMPs provide a new prothrombotic interface for fibrin, blood cells, and a growing thrombus resulting in narrowing of the vessel. The elevated shear stress due to vascular narrowing, protract this process by supporting further platelet activation and PMP release. Ultimately, an occlusive thrombus forms and patients go through grievous events. The PMPs contain receptors (GPIIb/IIIa, Ib, Ia, and IIa) for platelet–subendothelium attachment, and P-selectin for platelet–leukocyte interactions and inflammatory response [30]. Several studies have evidenced that circulating MPs might serve as potential prognostic markers for atherosclerotic vascular disease. PMPs that express P-selectin and CD63 on their surface are a sign of platelet activation in peripheral arterial disease and myocardial infarction (MI). A recent study demonstrated enhanced levels of PMPs in survivors of MI. It was shown that there is a considerable connection of large PMPs with plasma

thrombin–antithrombin complexes and soluble CD40 ligand in MI patients. Further, it has also been shown that interior and exterior diameters of carotid artery correlate inversely with circulatory MPs concentration [22].

3.3. Cancer

The involvement of high PMP levels in cancer metastasis cannot be ignored because it is linked to belligerent tumors and reduced response to treatment [31]. PMPs are reported to promote angiogenesis (which involves propagation, endurance, migration, and tube formation of human umbilical vein endothelial cells) during tumor growth via the combined action of vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and a lipid factor [32]. The pro-angiogenic effect of PMP is brought about by PI3-kinase and ERK pathways. It is thought that the PMP concentration at the sites of tumors may be unusually high. In gastric cancer, PMP levels are better forecasters of metastasis than plasma levels of IL-6, RANTES, and VEGF [33]. PMPs can also trigger the secretion of matrix metalloproteinase MMP-2 in prostate cancer cells, promoting tumor invasiveness by their easy passage through collagen, a chief constituent of the extracellular matrix [34]. Thereby, the generation of PMPs over an extended period can be lethal, and it is imperative to assess their count not only during disease conditions but also during chemotherapy. In fact, it has been reported that chemotherapy can cause platelet activation and MP generation, which in turn enhances the risk of thrombosis. The MPs express adhesion molecules and release growth factors and MMPs, all of which are essential for angiogenesis. These MPs interact with tumor cells and their environment, and further aid tumor growth. PMPs play a role in intercellular

cross-talk and express various proteins on their surface, including platelet–endothelium attachment receptors (CD41, CD61 and CD62), G-protein receptors (CXCR4 and PAR-1), cytokine receptors (TNF-RI, TNF-RII, CD95) and ligands (CD40L and PF-4), all of which can be transferred to the surface of tumor cells. Moreover, PMPs elicit mRNA expression of angiogenic factors such as MMP-9, vascular endothelial growth factor, hepatocyte growth factor and IL-8 [35].

3.4. Malaria

Malarial infection is associated with thrombocytopenia, as a result of shortened platelet lifespan. Thrombocytopenia is found to be associated with a concurrent increase in the number of MPs in plasma. Activated platelets and MPs are reported to play crucial roles in altering the blood–brain barrier leading to severe form of malaria called cerebral malaria [36].

3.5. Arthritis

Rheumatoid arthritis (RA) is an incapacitating systemic autoimmune inflammatory disease which mainly afflicts the synovial joints by evoking inflammation, and is characterized by immune cell recruitment and blood vessel dilation. It has been reported that platelets accumulate in the joints of RA patients and that elevated numbers of PMPs are found in their synovial fluid. Boilard et al. explored the involvement of platelets in RA and demonstrated the presence of PMPs in the synovial fluid from the patients. Further, the authors showed the pro-inflammatory nature of PMPs via the elicitation of cytokine responses (IL-1) from synovial fibroblasts [37]. Detailed studies have revealed that collagen receptor GP-VI and its associated gamma chain of the Fc receptor play a key role in eliciting PMP production in arthritis pathophysiology. Further, fibroblast-like cells lining the joint cavity trigger the shedding of PMP. Consequently, the released PMPs interact with and activate fibroblast-like synoviocytes (FLS), which are important effector cells that mediate both immune activation and joint destruction. The stimulated FLS bring about inflammatory cytokine responses via IL-1. Thereby, the IL-1 packaged into the PMPs seems to play a principal role in amplifying inflammation. The elevated level of PMPs in RA patients hints at their possible role in the progression of CVDs as well. RA patients are more susceptible to cardiovascular mortality, suggesting its association with the degree of inflammation. These reports put forward that PMPs might be the culprits involved in the inflammatory and thromboembolic processes in RA patients [38].

3.6. Diabetes

Diabetes mellitus (DM) patients are reported with platelet hyper-reactivity and increased platelet activation due to a multitude of factors such as hyper-secretion of insulin,

hyperglycemia, hyperlipidemia, oxidative stress, endothelial dysfunction, and inflammatory condition. Hyperglycemia, the basic characteristic feature of DM can cause increased platelet reactivity and platelet activation as indicated by markers such as soluble P-selectin and CD40-ligand, which in turn might be due to protein kinase C (PKC) activation, a transduction pathway regulator of proaggregatory platelet agonists. Platelets from DM patients also exhibit short-term activation of the calcium-sensitive PKC β isoenzyme. Moreover, the advanced glycation end products (AGEs) that are produced in excess in DM patients can cause PS externalization in platelets leading to surface clotting factor activation, thus augmenting the thrombogenic state. The surface membrane proteins also undergo enhanced glycation, which causes decreased membrane fluidity and increased platelet sensitivity to agonists. DM patients have evidences of increased oxidative stress and inflammation as well [39]. Cohen et al. reported that there is a drastic increase in thrombin activity in the type 2 diabetic blood along with a significant increase in PMPs. Furthermore, increased activities of caspases-3, -6, and -8 were also reported. Thus, it was conjectured that the increased thrombin activity, PMP formation and caspase activity together may contribute to the hypercoagulability of diabetic blood. The study highlighted the link between diabetic platelets and coagulation proteins that lead to pro-thrombotic condition and validates the increased risk of thromboembolic events in diabetic population [40]. PMPs were also reported to promote the expression of adhesion molecules in monocytes and endothelial cells and therefore, it appears that they might even participate in the development of atherosclerosis in diabetics [41]. Furthermore, a recent study uncovered the molecular pathway for platelet mitochondrial damage in DM, wherein it was shown that hyperglycemia leads to activation of aldose reductase ensuing in ROS generation, with consequent increase in p53 phosphorylation. These events were reported to cause loss of function and damage to mitochondria, mitochondrial membrane potential dissipation, cytochrome c release, caspase-3 activation and PS exposure, thus resulting in platelet apoptosis [42]. Although this study highlights the potential of aldose reductase inhibitors in DM treatment, it does not dwell into the aspect of aldose reductase-induced PMP generation in DM and its consequences.

3.7. Hyperthermia and dengue

Hyperthermia-related ailments such as dengue fever and heatstroke are often associated with hemorrhage and reduced platelet count. Therefore, Wang et al. investigated the effect of hyperthermia on platelet physiology and found that hyperthermia triggers apoptotic events in platelets, including $\Delta\Psi_m$ depolarization, caspase-3-dependent gelsolin cleavage and PS exposure, in addition to shedding of platelet glycoprotein Ib α ectodomain [43]. Further, it was reported that mitochondrial ROS play a vital role in hyperthermia-induced platelet apoptosis, and that the decrease in platelet

manganese superoxide dismutase activity might probably be responsible for the enhanced ROS levels in hyperthermia-induced platelets [44]. In a more recent study it was reported that in the course of dengue infection activated and apoptotic platelets aggregate with monocytes and trigger specific cytokine responses including the production and secretion of IL-1b, IL-8, IL-10, and MCP-1 that may play a role in the disease pathogenesis [45]. Though the role of platelets in monocyte responses was clearly demonstrated in this study, the possibility of PMS interacting with monocytes to further aggravate the inflammatory and immunomodulatory reactions during dengue infection needs further investigation.

3.8. Alzheimer's disease

Alzheimer's disease (AD) is an age-related neurodegenerative condition characterized by the cerebral amyloid angiopathy (CAA), which involves the deposition of neurotoxic amyloid- β (A β) plaque in brain parenchyma and cerebral blood vessels. Platelets contain the enzymatic machinery to produce A β peptides; therefore, platelets are not only acknowledged as *ex vivo* model to explore the pathophysiology of AD, but also regarded as biomarkers for the initial diagnosis of AD [46]. A recent study by Gowert et al. demonstrated that A β -treated platelets exhibit apoptosis-like events including ROS generation, cytosolic Ca²⁺ activity, mitochondrial membrane depolarization, caspase-3 activation, cell shrinkage and PS externalization. It also showed the vital role played by platelets in the development of CAA via release and modulation of A β into soluble fibrillar structures that were absorbed by apoptotic platelets. These events enhanced platelet adhesion and accumulation at amyloid deposits of cerebral vessels leading to thrombus formation and vessel occlusion, which are decisive for strokes in AD patients [47]. However, the study does not probe the role of PMPs released from A β -induced apoptotic platelets in the pathophysiology of AD.

4. Effect of pro-apoptotic biologicals on platelets

Numerous studies of late have reported drug-induced platelet apoptosis, a fact that was not known till a decade ago. At present, various commonly used biologicals such as, melatonin, vancomycin, balhimycin, cisplatin, arsenic trioxide, aspirin, resveratrol, sesamol, dibucaine, andrographolide, gossypol and carmustine are reported to elicit platelet apoptosis (Table 1) [6,7,11,12,48–52].

5. Platelet anti-apoptotic biologicals and their application in auxiliary therapy

Even though numerous studies that have demonstrated the wide array of protective efficacies of phytochemicals, there are only two reports hitherto suggesting the platelet

anti-apoptotic effects viz. cinnamtannin B1 and crocin. Cinnamtannin B1 (CTB), a proanthocyanidin is found in many species such as *Laurus nobilis* L. and *Cinnamomum verum*. Proanthocyanidins including CTB have been widely reported to display anticancer property via selective proapoptotic action in a wide range of cancer cell lines at the same time exerting antiapoptotic effects on healthy cells. Bouaziz et al. investigated the effects of CTB on platelets and found that it impaired thrombin-evoked activation of caspase-3 and -9 as well as their translocation to the cytoskeleton. CTB also inhibited thrombin-induced PS externalization and endogenous generation of H₂O₂, as well as H₂O₂-induced caspase activation and PS exposure. The concentration of CTB used was similar to that used to induce apoptosis in cancer cells [53]. Crocin is a carotenoid found in the stigma of flowers of *Crocus sativus* L. (saffron). Crocin reportedly possesses various health-promoting properties such as, anticarcinogenic, antihyperlipidemic, antiarthritic and hepatoprotective properties. Thushara et al. demonstrated that crocin protects platelets from oxidative stress-induced apoptosis. It ameliorates various events of the H₂O₂-induced intrinsic apoptotic pathway including $\Delta\Psi_m$ depolarization, cytochrome c translocation, caspases 9 and 3 activation, PS scrambling, ROS generation and Ca²⁺ mobilization. Besides, it was also found to inhibit agonist-induced platelet aggregation [54].

6. Future directions and conclusion

Platelets are now known to affect a wide array of physiological and pathological processes. Moreover, the platelet-derived MPs under normal physiological status are also known to play pivotal roles in a wide array of physiological functions and also aid in the maintenance of homeostasis. The sensitive nature of platelets makes them easily susceptible to various stimuli that can trigger apoptosis at an exacerbated rate. This can result in severe reduction of platelet count and thus affect the normal physiological processes that involve platelets. Several scientific studies of late, have reported the pro-apoptotic tendency of commonly used biologicals. Besides, the excess PMPs that are produced in the melee may have consequences of pathological order. With their rich content of cytosolic and surface molecules, which include a wide variety of cytokines and growth factors, PMPs are capable of simultaneously triggering multiple signaling systems and thus when they are produced in excess can have off-shoot reactions as well [55]. This may result in a vicious cycle wherein a platelet-proapoptotic drug used to treat one disease, which for the time-being might cure the condition, but may later cause the re-emergence of the same disease with stronger symptoms or may lead to another disease. Obviously, the system is subjected to more drugs, resulting in more stress. For instance, there are several reports of recurrence of tumor at same sight or at different one, increased risk of CVDs in arthritis and

Table 1

List of biologicals that induce platelet apoptosis and their respective mechanism of action.

Biological	Therapeutic indications	Reported pro-apoptotic events in platelets
Vancomycin	In the treatment of gram-positive penicillin-resistant infections, bacterial endocarditis in penicillin-allergic patients, MRSA and medical device-associated infections	Phospholipid scrambling, PS externalization, decrease in platelet volumes, increase in intracellular Ca^{2+} , $\Delta\Psi_m$ depolarization, ceramide formation, and caspase-3 activity
Balhimycin	Treatment of MRSA	Cell shrinkage, cell membrane scrambling, increase in intracellular Ca^{2+} , ceramide formation, $\Delta\Psi_m$ depolarization and caspase-3 activity
Cisplatin	Treatment of tumors of the brain, breast, cervix, liver, lungs, head and neck, ovaries, testes etc. as well as osteosarcoma, melanoma, neuroblastoma and non-Hodgkin's lymphoma	ROS production, up-regulation of proapoptotic Bax and Bak, and mitochondrial translocation of Bax, down-regulation of antiapoptotic Bcl-2 and Bcl-XL, increase in intracellular Ca^{2+} , $\Delta\Psi_m$ depolarization, calpain activation, caspase-3 activation, and PS exposure via extracellular signal-regulated protein kinase (ERK) signaling pathway
Aspirin	As antiplatelet agents to treat thrombotic occlusion of coronary and cerebral arteries, and vascular grafts; administered immediately after MI to reduce the risk of another attack and revive the cardiac tissue	$\Delta\Psi_m$ depolarization, PS exposure, caspase-3 activation, and platelet shrinkage
Arsenic trioxide	Treatment of acute promyelocytic leukemia, lymphoma, hepatocellular carcinoma, myelodysplastic syndrome	$\Delta\Psi_m$ depolarization, up-regulation of Bax and down-regulation of Bcl-2 and Bcl-XL, caspase-3 activation, and PS exposure via JNK activation
Dibucaine	Local anesthetic used as a spinal and topical anesthesia	$\Delta\Psi_m$ depolarization, Bax and Bak up-regulation, Bcl-2 and Bcl-XL down-regulation, caspase-3 activation, and PS externalization
Resveratrol	Antioxidant, cardioprotective, cancer preventive, anti-diabetic, neuroprotective, anti-inflammatory effects	Cleavage of Bid into tBid, translocation of Bax into mitochondria, release of cytochrome c from mitochondria, $\Delta\Psi_m$ depolarization, activation of caspases-8, -9 and -3, cytoskeleton remodeling, cell shrinkage, and PS externalization
Thymoquinone	Antioxidant, cardioprotective, anti-cancer, hepatoprotective, renoprotective, analgesic and anticonvulsant properties	Stimulates platelet apoptosis via GPCR-activated PI3K pathway involving increase in intracellular Ca^{2+} , $\Delta\Psi_m$ depolarization, ceramide formation, caspase 3 activation, cell shrinkage and PS exposure
Gossypol	Anti-cancer effects	Up-regulation of Bax, coated platelet formation, $\Delta\Psi_m$ depolarization, PS exposure, and release of MPs
Andrographolide	Anticancer, antioxidant and hepatoprotective properties	$\Delta\Psi_m$ depolarization, activation of caspases-3 and -8, cleavage of Bid into tBid
Sesamol	Antiaging, antioxidant, hepatoprotective, neuroprotective, antiinflammatory, chemoprotective, chondroprotective and anti-arthritis properties	Elevated intracellular ROS particularly H_2O_2 and Ca^{2+} , $\Delta\Psi_m$ depolarization, cytochrome c release, activation of caspases-9 and -3, and PS externalization
Melatonin	Managing jet lag, insomnia, blood pressure, fatigue, ovarian physiology and fibromyalgia	Elevated intracellular ROS and Ca^{2+} , $\Delta\Psi_m$ depolarization, cytochrome c release, activation of caspases-9 and -3, protein phosphorylation, PS externalization, and loss of platelet viability
Carmustine	Hodgkin's and non-Hodgkin's lymphoma, brain cancers and multiple myeloma	$\Delta\Psi_m$ depolarization, up-regulation of Bax, down-regulation of Bcl-2 and caspase-3 activation

diabetic patients etc. Thus, the single-dimensional treatment regimen of targeting the symptoms is itself questionable, and rather a holistic approach of healing is required in today's time. In this perspective, the potential use of plant-derived biologicals as auxiliary therapy along with the normal treatment regimen could be considered. One more approach, which is rather futuristic, is to exploit MPs in disease management. It is a known fact that platelets produce different types of MPs in response to different stimuli under varying physiological and pathological conditions. These PMPs vary in their composition and thereby in their effect produced on the target cells as well. Therefore, with the background of recent research activities on personalized medicines, we hypothesize that the future of MPs would be their application in disease management. The negative side of MPs if it can

be said so, can be exploited in a positive manner by means of generating tailor-made MPs. If MPs could be generated by incorporating specific effector molecules and as well as surface antigens, which can target a specific set of cells or tissues, it could turn out to be a revolution in the new-age disease management. By virtue of their small size, the variety of cytosolic fractions that they can carry and their accessibility to the biological system so as to bring about a wide array of pathophysiological effects, makes them potential candidates in the future of therapeutic stratagem. Furthermore, it is a known fact that platelets are abundant sources of factors associated with wound healing such as platelet-derived growth factor (PDGF), transforming growth factor (TGF) and vascular endothelial growth factors (VEGF) [56]. It is the basis of PRP therapy for tissue repair and it

is being used in the treatment of osteoarthritis, bone repair and regeneration, tendinitis, nerve injury, cardiac muscle injury, etc. However, it has been reported that in stored PRP even under standard banking conditions, platelets eventually lose their activity and tend to undergo apoptosis [57]. Thus, it is obvious that there will be an increased concentration of MPs in stored platelet concentrates, which might lead to secondary complications if used for treatment. Hence, more research is required in this direction and the option of tailor-made MPs instead of PRP therapy could be explored.

Conflicts of interest

Authors have no conflict of interest to be declared.

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