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Zinc: an endogenous and exogenous regulator of platelet function during haemostasis and thrombosis

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Abstract

Zinc (Zn^{2+}) is an essential micronutrient and the second most abundant trace metal in the human body. The important role that Zn^{2+} plays in haemostasis is exemplified by platelet-related bleeding phenotypes coinciding with dietary Zn^{2+} deficiency. These phenotypes are rectified upon Zn^{2+} supplementation. Labile (unbound) Zn^{2+} is present in the plasma at micromolar levels, but is also detected in atherosclerotic plaques, and released from platelet alpha granules. Therefore, it is likely that localised Zn^{2+} concentrations are higher at sites of thrombosis and haemostasis. Exogenous Zn^{2+} is a regulator of the haemostatic responses, with roles during coagulation and platelet activation. Extracellular Zn^{2+} gains access to the platelet cytosol and induces full platelet activation at high concentrations, and potentiates platelets to activation by conventional agonists at lower concentrations. Zn^{2+}-induced platelet activation is dependent on PKC and integrin $\alpha_{IIb}\beta_3$, and is associated with tyrosine phosphorylation of platelet proteins. Agonist evoked platelet activation results in intracellular Zn^{2+} ($[Zn^{2+}]_i$) fluctuations that are sensitive to the platelet redox state. Increases in $[Zn^{2+}]_i$ correlate with activation responses, including shape change, granule release, $\alpha_{IIb}\beta_3$ activation and phosphatidyl-serine exposure, consistent with a role as a second messenger. This review provides insight into the numerous demonstrated and potential roles for Zn^{2+} in platelet function during thrombosis and haemostasis, highlighting its increasing acceptance as an intracellular and extracellular platelet regulatory agent.

Introduction

A central role for metal cations in the regulation of biological systems is well established. Zinc (Zn^{2+}) is a micronutrient, the second most abundant trace metal in the human body, and is recognised as an essential component for all forms of life.[1] Its importance and roles in numerous biological processes is exemplified by the pathological effects of dietary Zn^{2+} deficiency, which is responsible for 1.4% of deaths worldwide and is a leading cause of mortality and morbidity in the developing world.[2,3]

Zn^{2+} is present in plasma at concentrations ranging from 20-30μM, much of which is associated with plasma proteins including albumin and α2 macroglobulin, resulting in a free (or labile) concentration of 0.5μM.[4–8] Studies have correlated dietary Zn^{2+} intake or serum Zn^{2+} concentrations with cardiovascular disease (CVD) risk. Early work correlated inadequate Zn^{2+} intake with increased incidence of CVD.[9,10] However, this has been challenged by recent meta-analyses.[11] Correlation of plasma Zn^{2+} levels with CVD has produced mixed results. Dietary Zn^{2+} deficiency reduces plaque development in animal models.[12] However in humans, some studies indicate that Zn^{2+} deficiency contributes to atherosclerosis development,[13–15] whilst others have shown no correlation.[16] Zn^{2+} accumulates in atherosclerotic plaques to levels six-fold greater than surrounding tissues, potentially providing a reservoir that is potentially accessible to haemostatic processes following plaque rupture.[17,18]

Zn^{2+} has numerous essential roles in cellular biochemistry. Early research identified associations between Zn^{2+} and enzymes including carbonic anhydrase where Zn^{2+} forms a critical part of the catalytic site.[19] Subsequently, it was found that certain transcription factors utilise Zn^{2+} as a structural component, with Zn^{2+}-binding motifs commonly consisting of repetitive histidine- and cysteine-containing sequences. These motifs are one of the most common structural motifs in eukaryotic cells.[20] Using bioinformatics approaches, Zn^{2+} binding sites have been identified in more than 3000 different proteins, representing approximately 10-15% of the proteins within the human genome.[4,21] Recent work has established dynamic signalling roles for Zn^{2+} in different cell types, challenging the view that Zn^{2+} is an inert, structural cofactor. Zn^{2+} acts as both an intracellular and extracellular signal transduction molecule.[22–24] Increases in intracellular Zn^{2+} ($[Zn^{2+}]_i$) regulate responses in numerous cell types, establishing Zn^{2+} as a second messenger. Basal $[Zn^{2+}]_i$ in eukaryotic cells is at picomolar concentrations, rising to nanomolar upon stimulation.[25,26] In comparison, basal calcium $[Ca^{2+}]_i$ levels are in the nanomolar range, rising to micromolar. Despite this, Zn^{2+} frequently interacts with proteins at a higher affinity than Ca^{2+}, and is therefore a potent effector at lower concentrations.[27] Interestingly, many of the chelators, fluorophores, and ionophores utilised in Ca^{2+} research, have higher affinities for Zn^{2+}. Therefore it is highly likely that the role and importance of Zn^{2+} in biological systems is under-reported.

Zn^{2+} as a mediator of the haemostatic response

~~Zinc~~ Zn^{2+} is involved in wound healing and skin repair, as shown in patients with dietary Zn^{2+} deficiency, in whom wound healing is delayed.[28–30] High concentrations of Zn^{2+} are present in the dermal and epidermal tissue from where it is released following damage.[31] Zn^{2+} increases in the early inflammatory phase of experimental wounds,[32–34] and in rodent skin wound models Zn^{2+} levels at the wound margin are increased by 15-20% within 24 hours.[30] This increase in Zn^{2+} may be attributable to recruitment of cells with high Zn^{2+} content (including erythrocytes, neutrophils lymphocytes and platelets) and may represent a mechanism by which Zn^{2+} can be delivered to sites of vascular damage.[35]Whilst not yet confirmed experimentally, it is possible that Zn^{2+} concentrations are elevated in the vicinity of vascular injury, following plaque rupture, or within a growing thrombus, resulting in Zn^{2+}-rich microdomains that may regulate haemostatic responses. Indeed, both platelets and coagulation factors are sensitive to increases in Zn^{2+} concentration, indicating a role for Zn^{2+} in coagulation and thrombosis.

The role of Zn^{2+} as a haemostatic regulator is evident in models of dietary Zn^{2+} deficiency. Zn^{2+}-deficient rodents have an increased bleeding tendency, more difficult parturition, and prolonged tail bleeding time.[36–39] These deficiencies correlate with platelet-related effects, exemplified by reduced responses to agonists, thromboxane A2 generation, and levels of Inositol triphosphate (IP3).[38,40–42] Similar responses are observed in humans, where Zn^{2+} deficiency correlates with impaired platelet aggregation.[39] Interestingly, ecchymosis, prolonged bleeding times, and abnormal platelet aggregation responses were observed in two cancer patients with dietary Zn^{2+} deficiency.[43] In both cases, the bleeding phenotype was normalised following oral administration of ~~zinc~~ Zn^{2+} sulphate, which could be reversed by discontinuation of therapy.

~~Zinc~~ Zn^{2+} homeostasis, storage and release in platelets and megakaryocytes

Zn^{2+} is present in platelets, where it contributes to homeostasis and activatory processes during thrombus formation. It is also present in megakaryocytes, although its role in these cells is poorly understood (see Mammadova-Bach and Braun, 2019 for a recent review,[44]) $[Zn^{2+}]_i$ can be present in a protein-bound form, or as a free (or 'labile') Zn^{2+}. Cellular Zn^{2+} influx and efflux is coordinated by members of the ZIP and ZnT family of proteins which act as active Zn^{2+} transporters.[45–47] Family members are generally selective for Zn2+, although some family members have been shown to transport Fe^{2+}, Mn^{2+} or Cd^{2+}.[48] mRNA profiling of megakaryocytes indicates that ZnTs, 1, 5, 6, 7, 9, and most of the ZIP family members are expressed,[49] whilst proteomic data provide evidence for ZIP7, ZIP3, and ZnTs 1, 5, and 6 in human platelets.[50] In nucleated cells, ZIP7 and ZnTs 1, 5, 6 and 7 reside on organelle membrane where they regulate efflux of Zn^{2+} from the cytosol to intracellular organelles. ZIP7 is expressed on the ER and Golgi,[45,51,52] where it participates in cell proliferation and migration.[53] ZIP3 is sensitive to $[Zn^{2+}]_i$, which result in its translocation to the cell membrane where it regulates Zn^{2+} entry.[54–56] $[Zn^{2+}]_i$ fluctuations are buffered by Zn^{2+}-binding proteins, of which metallothioneins (MTs) are the best understood.[57–59] In nucleated cells, Zn^{2+} buffering occurs as a result of cytosolic redox changes, which liberate Zn^{2+} from binding sites. MTs are present in the platelet and megakaryocyte cytosol where they may perform a similar role.[57,58] Whilst the role of MTs in platelet function is poorly understood, incubation of platelets with MT reduces signs of platelet activation.[58]

Release of Zn^{2+} upon platelet degranulation

Degranulation following platelet activation is a critical aspect of the haemostatic response, contributing to coagulation, wound healing and inflammation.[60] The α granule represents a major Zn^{2+} store in platelets,[61–63] where Zn^{2+} is likely to associate with Zn^{2+}-binding proteins including fibrinogen, FXIII, and histidine-rich glycoprotein (HRG). Recently, the Zn^{2+} fluorophore Fluozin-3 (Fz3) was used to visualise Zn^{2+} in α granules.[49] Thrombin stimulation decreased the Fz3 signal in wild type platelets, whilst platelets from $NBeal2^{-/-}$ mice had reduced fluorescence. $Unc13d^{-/-}$ mouse platelets had a moderately reduced fluorescence, which was not further reduced in response to thrombin.[49] These data are consistent with Zn^{2+} being packaged into α granules and released upon degranulation, contributing to increased concentrations of local extracellular Zn^{2+} ($[Zn^{2+}]_e$, Figure 1). Zn^{2+} concentrations at sites around vascular injury, or within a growing thrombus are difficult to determine. Zn^{2+} concentrations following stimulation of $3.5x10^9$/mL platelets with collagen was estimated to be approaching 3nmol in a 100µL volume (30µM).[64] At these concentrations, $[Zn^{2+}]_e$ has the potential to affect a number of haemostatic processes, including coagulation (reviewed elsewhere).[65] The role of Zn^{2+} in fibrin formation following platelet degranulation has been explored using perfusion-based models of thrombus formation.[49] Reduced fibrin formation was observed in blood from $Nbeal^{-/-}$ or $Unc13d^{-/-}$ mice. This was partially restored upon addition of Zn^{2+}, confirming that Zn^{2+} release during degranulation is an important feature of the coagulation response. Confirmatory work has been performed using platelets from Hermansky-Pudlak Syndrome (HPS) and Gray platelet syndrome (GPS) patients.[49]

Increased $[Zn^{2+}]_o$ induces platelet activation

In addition to affecting coagulation, increase $[Zn^{2+}]_o$ at sites of vascular injury is likely to affect platelet responses. Zn^{2+} activates platelets *in vitro*, with high concentrations (100µM) being able to induce full platelet activation.[66–68] Sub-activatory levels of Zn^{2+} (30µM) potentiate platelet aggregation in response to threshold concentrations of collagen, ADP, U46619, thrombin and adrenaline.[66,69] As 30µM is not considerably higher than the 0.5-1µM free Zn^{2+} found in the blood, it is feasible that these levels could be attained as a result of vascular injury, atherosclerotic plaque rupture, or platelet degranulation. In support of this observation, chelation of intracellular Zn^{2+} ($[Zn^{2+}]_i$) with TPEN (N,N,N',N'-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine) prevents the potentiating effect.[66,67] In an *in vitro* flow model, Zn^{2+} chelation reduces activatory responses, without affecting adhesion.[66] Whilst it is not known whether TPEN chelates labile or protein-bound $[Zn^{2+}]_i$, these data demonstrate a clear role for $[Zn^{2+}]_i$ in platelet activation.

Incubating FZ-3-loaded platelets with Zn^{2+} results in increases in fluorescence, consistent with entry of Zn^{2+} into the cytosol.[66] The entry mechanism remains an area of active research. Pre-treatment with TRP channel inhibitors, 2-APB, flufenamic acid, or the reverse mode NCX inhibitor KB-R resulted in significant decreases in Zn^{2+} fluorescence, implicating TRP channels and the NCX exchanger in Zn^{2+} entry (unpublished observation). Co-treatment with 2-APB and KB-R caused a further reduction in Zn^{2+} entry, suggestive of multiple routes for Zn^{2+} entry (Figure 1). Further investigation is required to confirm Zn^{2+} entry routes in platelets.

Upon entry, exogenous Zn^{2+} modulates platelet behaviour and influences signalling processes leading to platelet activation in a manner that is consistent with a conventional platelet agonist.[66,67,69,70] Zn^{2+}-induced activation, is dependent on PKC activity, upon which Zn^{2+}-binding sites have been identified.[71,72] PKC is influenced by changes in $[Zn^{2+}]_i$, as Zn^{2+} enhances association of PKC with the cytoskeleton of cell homogenates and phorbol esters.[73] Zn^{2+}-induced activation is sensitive to pre-treatment with $\alpha_{IIb}\beta_3$ inhibitors, indicating a dependence on $\alpha_{IIb}\beta_3$ activation, consistent with conventional platelet activatory responses.[66]

~~Zinc~~ Zn^{2+} acts as an intracellular second messenger in platelets

Zn^{2+} stores and agonist-dependant $[Zn^{2+}]_i$ fluctuations have been observed in nucleated cells. Increases in Zn^{2+} have been correlated with changes in cellular responses, including binding to, and regulation of intracellular proteins. This is consistent with a role for Zn^{2+} as an intracellular second messenger.[74] In nucleated cells, agonist stimulation results in Zn^{2+} release from intracellular organelles via both Zn^{2+} transporters, and from metallothioneins.[74–78] Other recognised examples of Zn^{2+} acting as a second messenger include in mast cells, where extracellular stimulation of the Fcε receptor results in intracellular Zn^{2+} ($[Zn^{2+}]_i$) elevation evoking a 'Zn^{2+} wave', which is followed by modulation of tyrosine phosphatase activity.[23] Monocytes also undergo $[Zn^{2+}]_i$ elevation upon stimulation, further illustrating the role of Zn^{2+} as an intracellular messenger.[24] Agonist-evoked fluctuations of $[Zn^{2+}]_i$ have been shown to modulate signalling proteins (including PKC CamKII, and IRAK) in a similar manner to Ca^{2+}.[70,72,74]

Recent work from our laboratory has demonstrated that agonist stimulation of platelets results in $[Zn^{2+}]_i$ fluctuations, and that increases in $[Zn^{2+}]_i$ correlate with changes to platelet function.[79] CRP-XL or U46619 (GpVI and TPα ligands respectively) evoked increases in $[Zn^{2+}]_i$ with GpVI stimulation producing a larger effect.[79] Interestingly, thrombin stimulation did not yield $[Zn^{2+}]_i$ increases. This observation is difficult to reconcile with TPα and PAR sharing similar signalling pathways, but may be attributable to stronger responses to thrombin compared to TPα. Agonist-evoked $[Zn^{2+}]_i$ increases are distinct from $[Ca^{2+}]_i$ signals, and are sensitive to pre-treatment with TPEN, or by antioxidants, indicating that redox-sensitive proteins may act as Zn^{2+} stores. Confirmation of a role for Zn^{2+} as a second messenger requires evidence linking $[Zn^{2+}]_i$ fluctuations with changes in platelet function. The effect of increased $[Zn^{2+}]_i$ in platelets has been investigated using ~~**Effects of increased $[Zn^{2+}]_i$ on platelet responses**~~

~~The influence of increases in $[Zn^{2+}]_i$ on functional responses have been investigated using~~ Zn^{2+} ionophores, of which Clioquinol (Cq) and Pyrithione (Py) are widely used in ~~zinc~~ Zn^{2+} research in nucleated cells.[79] These show significant specificity for Zn^{2+} over other cations, whilst other ionophores used to model Ca^{2+} increases are less specific. The Ca^{2+} ionophore A23187 for example has high affinity for a wide variety of cations, raising the question as to whether accepted Ca^{2+} ionophore responses are primarily attributable to Ca^{2+} or Zn^{2+}.[79–81] Treatment of platelets with Py or Cq results in increases in $[Zn^{2+}]_i$, with Cq producing a greater response.[79] $[Zn^{2+}]_i$ increases correlate with platelet activation. Cq induces shape change and aggregation, whilst responses to Py are limited to shape change (Figure 1). Aggregation responses to Cq are dependent on PKC and $\alpha_{IIb}\beta_3$ activity (unpublished observation). Consistent with a role for $[Zn^{2+}]_i$ in aggregation is the observation that Cq treatment results in increased PAC-1 binding, confirming $\alpha_{IIb}\beta_3$ activation.[79] Zn^{2+}-ionophore-mediated shape change is sensitive to cytochalasin-D pretreatment.[79] Furthermore, reductions in platelet spreading on fibrinogen are observed in TPEN-treated platelets, and ionophore treatment is accompanied by myosin light chain (MLC) phosphorylation, and VASP dephosphorylation, both indicators of platelet shape change responses.[79] These observations identify $[Zn^{2+}]_i$ fluctuations as being central to platelet shape change, consistent with

observations in nucleated cells.[82–84] Platelet shape change occurs in a Ca^{2+}-independent manner, where p160 ROCK (Rho-associated protein kinase) either phosphorylates MLC and/or enhances MLC phosphorylation by inhibiting MLC phosphatases (MLC-P).[85,86] Inhibition of MLCK and p160 ROCK results in similar levels of inhibition of shape change in response to Zn^{2+} ionophores (unpublished observation). Conversely, inhibition of calmodulin did not significantly influence Zn^{2+} ionophore-induced shape change (unpublished observation). This is consistent with a role for Zn^{2+}-induced shape change that is independent of Ca^{2+} dependent signalling pathways. Previous studies have shown that the Rho-binding domain of p160 ROCK consists of a cysteine-rich Zn^{2+} finger-like motif, although the contribution of this motif to p160ROCK activity has not been confirmed. Zn^{2+} has a role in regulating hypoxia-induced MLC phosphorylation which was found to be ROCK1 dependent, which similarly consists of a Zn^{2+}-binding motif.[87,88] These studies suggest a mechanism by which Zn^{2+} evokes p160 induced platelet shape change, as Zn^{2+} may regulate p160 ROCK activity by binding to the Zn^{2+} binding motif found within this kinase.

In nucleated cells, $[Zn^{2+}]_i$ fluctuations are correlated with apoptosis, which includes phosphatidyl-serine (PS) exposure. In platelets, PS exposure is an activation-dependent response in a subset of platelets, providing a matrix for the initiation of coagulation. Whilst the importance of Ca^{2+} in PS exposure in platelets is well established, the role of Zn^{2+} less clear.[89,90] Increasing $[Zn^{2+}]_i$ using ionophores results in increased PS exposure.[79] This effect is sensitive to TPEN, and occurs at a reduced rate compared to A23187, indicating that $[Ca^{2+}]_i$ plays a predominant role. Cq-mediated PS exposure is partially sensitive to the caspase inhibitor Z-VAD, indicating a limited role for caspases. Stimulation with U46619 or CRP-XL results in PS exposure in a manner that is sensitive to TPEN treatment, in contrast to thrombin-induced PS exposure which was TPEN insensitive. This is consistent with data showing that stimulation via GpVI or Tpα results in Zn^{2+} signals, whereas thrombin stimulation does not.[79] Ca^{2+} regulates PS exposure through activation of PKC, and also by activation of the lipid scramblase, TMEM16F.[96,97] As Zn^{2+} is acknowledged to be able to associate with Ca^{2+}-binding sites, there is a possibility that Zn^{2+} may regulate TMEM16F activity. Furthermore, TMEM16F acts as a non-selective channel during lipid scrambling, and therefore may play a role in Zn^{2+} translocation[93]

Increasing $[Zn^{2+}]_i$ using ionophores results in the externalisation of CD63, but not CD62P, supporting a role for Zn^{2+} in regulating dense, but not α granule release.[79] Similarly, TPEN abrogates agonist-dependent granule release. Distinct, but poorly understood signalling pathways contribute to differential release of platelet granules, and while the exact mechanism is poorly understood, this work provides evidence for a role for Zn^{2+} in dense granule secretion.[60,94]

Whilst the mechanisms of Zn^{2+} regulation of PS exposure, dense granule release and $\alpha_{IIb}\beta_3$ upregulation are yet to be determined, both exogenous Zn^{2+} treatment and ionophore-mediated increases in $[Zn^{2+}]_i$ results in phosphorylation of a number of cytosolic proteins.[66] Therefore, it is likely that differences in kinases or phosphatases are implicated in Zn^{2+}-dependent responses. Increases in tyrosine phosphorylation of proteins following Zn^{2+}-induced activation differs to that induced by CRP-XL or thrombin, indicating the involvement of a novel signalling pathway.[66] Interestingly, increased phosphorylation of a high molecular weight protein (approximately 95kDa) is observed upon chelation of $[Zn^{2+}]_i$ with TPEN, suggesting that Zn^{2+} may influence tyrosine kinase or phosphatase activity.[66] The pattern of phosphorylated proteins induced by Py and Cq are similar, although Cq induced greater phosphorylation of higher molecular weight proteins, suggesting involvement by kinases or phosphatases may be influenced by $[Zn^{2+}]_i$ (unpublished observation). Protein tyrosine phosphatases (PTP) positively and negatively regulate platelet activity, some of which are inhibited by Zn^{2+}.[95] Many platelet phosphatases such as PTP1B, SHP-1, SHP-2 and PTEN which regulate platelet activation, have IC_{50} values ranging in the nM to μM for Zn^{2+}, and are therefore susceptible to physiological $[Zn^{2+}]_i$ fluctuations.[70,72,96–101].

Zn^{2+} mediated activation of nucleated cells is associated with MAPK activation.[102–109] In platelets, Cq treatment increases phosphorylation of both Erk1/2 and JNK.[110] A23187 did not affect Erk1/2 or JNK responses, and TPEN pre-treatment abrogates agonist-evoked increases in phosphorylation, confirming a role for Zn^{2+} and not Ca^{2+}. MAPK activity regulates a number of platelet responses, including $\alpha_{IIb}\beta_3$ activation, granule release, thromboxane generation, and cytoskeletal rearrangements.[111,112] In nucleated cells $[Zn^{2+}]_i$ fluctuations and MAPK activity are associated with the generation of reactive oxygen species (ROS) and regulation of the redox state.[113–115] Platelets produce ROS following agonist stimulation, and anti-oxidant treatment reduces platelet responsiveness.[116] Additionally, platelets undergo activation in response to extra- and intracellular increases in ROS.[117,118] We have recently investigated the relationship between ROS generation and $[Zn^{2+}]_i$ in platelets.[110] Treatment with exogenous Zn^{2+} results in increases in ROS production, which are sensitive to NADPH oxidase inhibitors and mitochondrial activity. Furthermore, agonist-evoked increases in ROS are sensitive to $[Zn^{2+}]$i chelation. Both agonist-and ionophore-mediated increases in ROS are accompanied by reduction of reduced glutathione and glutathione peroxidase activity. GSH buffers intracellular ROS

formation through the reaction with glutathione peroxidase (GPx) and glutathione reductase (GR) to generate oxidized glutathione (GSSG), a marker of oxidative stress.[119] Furthermore, mimicking ROS generation using H_2O_2 resulted in increases in $[Zn^{2+}]_i$, suggestive of a feed-forward loop in which increases in $[Zn^{2+}]_i$ stimulate ROS generation, leading to further $[Zn^{2+}]_i$ release, perhaps from redox-sensitive proteins such as metallothioneins. An interrelationship between ROS and Zn^{2+} is supported by the observation that differences in $[Zn^{2+}]_i$ responses to agonist stimulation may be attributable to differences in ROS generation. GpVI stimulation results in greater levels of ROS generation compared to thrombin or U46619,[120–125] consistent with GpVI being the most potent stimulator of $[Zn^{2+}]_i$ responses. Platelets express both NOX1 and NOX2.[125–127] Whilst NOX1 is associated with PAR and TP activation, NOX2 is associated with GpVI activation.[128] Therefore it is possible that isoform differences are responsible for differing Zn^{2+} responses.

Another mechanism by which $[Zn^{2+}]_i$ fluctuations may effect platelet activity is in the regulation of cyclic nucleotide signalling. In nucleated cells, $[Zn^{2+}]_i$ fluctuations affect both adenylate cyclase (AC) and phosphodiesterase (PDE) activity, possibly by direct interaction with Zn^{2+} binding sites.[129–131] In PC12 cells for example, forskolin-evoked cAMP elevation is abrogated following treatment with extracellular Zn^{2+} or Py, suggesting that increases in $[Zn^{2+}]_i$ regulate cAMP-dependent signalling.[132] Whilst unstudied in platelets it is possible that $[Zn^{2+}]_i$ fluctuations have similar effects in platelets.

Concluding Statement

Whilst research into the relationship between Zn^{2+} and platelet behaviour is still in its infancy, evidence exists for important roles for Zn^{2+} as a haemostatic regulator following degranulation, as a platelet agonist, and also as an intracellular regulator of platelet responses. In intracellular signalling, the considerable range of functions in which Zn^{2+} participates, suggests that $[Zn^{2+}]_i$ fluctuations are central to the regulation of platelet behaviour. A scheme detailing the many potential influences of Zn^{2+} in platelet function is shown in Figure 1. However, whilst the role of Zn^{2+} in platelets is becoming more established, important questions remain. For instance, whilst it is know that they are redox sensitive, the nature of the Zn^{2+} store in platelets is yet to be determined. Furthermore, additional work dissecting the relative importance of Ca^{2+} and Zn^{2+} in platelet responses is also important. Despite this, it is becoming clearer that Zn^{2+} plays an important role in platelet behaviour during haemostasis and thrombosis, and should be considered when developing novel anti-thrombotic strategies.

FIGURE LEGENDS

Figure 1: Schematic showing the many potential roles of Zn^{2+} in platelet function.

Platelets respond to vascular injury by interaction with subendothelial matrix proteins, such as collagen (1) resulting in signalling through GpVI (1). Secondary agonists such as thromboxane contribute to signalling which results in Zn^{2+} release from intracellular stores (2). Receptor signalling leads to increases in ROS generation (3) resulting in the reduction of thiols on Zn^{2+}-binding proteins, such as metallothioneins (MT), leading to Zn^{2+} release (5). Cytosolic $[Zn^{2+}]_i$ increases (5), regulating further ROS production via NOX and mitochondrial activity (6). This generates a feedforward loop leading to further ROS production which is regulated by Erk1/2 and JNK phosphorylation. Increased $[Zn^{2+}]_i$ activates PKC (7) which regulates further platelet responses including cytoskeletal rearrangements leading to platelet shape change (8) RHO etc, and (9) PS externalisation. Increased $[Zn^{2+}]_i$ also results in degranulation of dense, but not alpha granules (10). Agonist stimulation results in alpha granule release (11), independently of Zn^{2+} signalling. $[Zn^{2+}]_e$ increases at sites of vascular injury as a result of platelet degranulation, release from damaged cells, and atherosclerotic plaque rupture (12). $[Zn^{2+}]_e$ gains access to the platelet through TRP channels and the NCX exchanger, working in reverse mode, further contributing to $[Zn^{2+}]_i$ increases. $[Zn^{2+}]_i$ inhibits adenylate cyclase, reducing cAMP levels and promoting platelet activation (13), and also inhibits phosphatases, enhancing tyrosine phosphorylation of signalling proteins (14). Created with BioRender.com.

Conflict of interest

The authors report no conflicts of interest.

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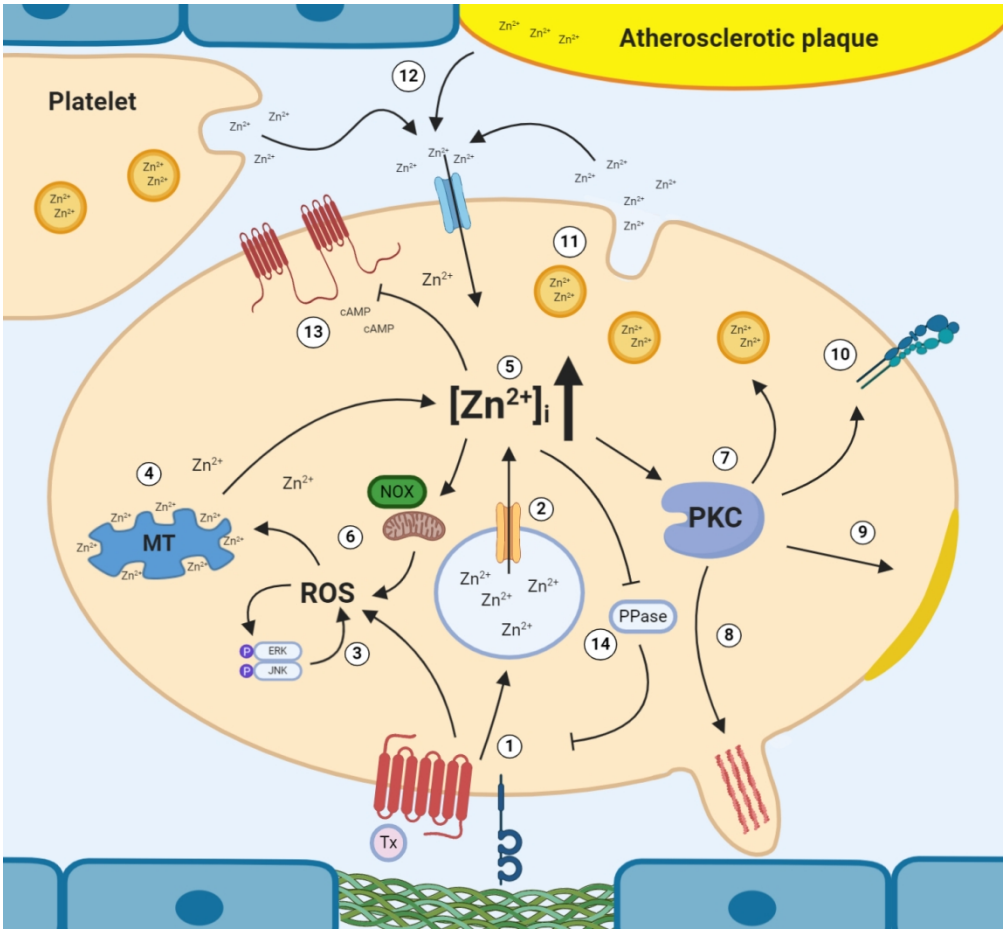
Atherosclerotic plaque
Platelet
12
11
13
cAMP
cAMP
5
$[Zn^{2+}]_i$
4
MT
6
NOX
ROS
2
7
PKC
9
10
8
PPase
14
3
ERK
JNK
1
Tx