Metabolism and Translational Medicine

REVIEW ARTICLE

Platelet metabolic remodeling in thrombosis

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ABSTRACT

Many human diseases are associated with hyperactivity of platelet metabolism. Understanding in detail the connection between metabolic changes and platelet activation state is an indispensable part of thrombosis treatment. This review describes changes in the energy metabolism of platelets during thrombosis and their potential molecular mechanisms. The metabolic flexibility of energy substrate selection in activated platelets is discussed briefly, along with strategies by which platelet activation and thrombosis formation can be blocked by targeting activated platelet metabolism.

Key words: thrombosis, platelets, glycolysis, fatty acid metabolism, amino acid metabolism

INTRODUCTION

Platelets play a vital role in the occurrence, development, and stability of thrombosis. Endothelial damage triggers platelet activation, adhesion, and aggregation, thereby promoting rapid hemostasis. In contrast, inappropriate platelet activation promotes vasculitis, formation of atherosclerosis, and arterial thrombosis.[1,2] Therefore, targeting platelets is one of the main anti-thrombotic strategies, with antiplatelet drugs being the cornerstone of treatment for arterial thrombotic diseases such as acute myocardial infarction and ischemic stroke. However, currently available antiplatelet drugs have always had shortcomings, such as severe bleeding and insufficient efficacy.[3] This suggests that the mechanisms of platelet activation are largely still unknown. Platelets are anuclear, meaning they do not replicate, nor can they respond to environmental cues by changing the levels of messenger ribonucleic acid.[4] Therefore, activated platelets must quickly adjust their metabolism, which is very important. The transition from a resting to an activated state in platelets leads to a huge change in the demand for adenosine triphosphate (ATP).[5,6] Recently, changes in flux through different metabolic pathways have been shown to affect the formation of thrombosis. Here, we briefly discuss the metabolic changes that occur in platelets during thrombosis and describe new methods of inhibiting thrombosis formation by targeting platelet metabolism.

ENERGY METABOLISM IN RESTING PLATELETS

Platelets primarily obtain the required ATP through mitochondrial oxidative phosphorylation and glycolysis. In resting platelets, nearly 60% of ATP is produced by glycolysis, with oxidative phosphorylation providing the remaining 40%.[7] Resting platelets also exhibit "metabolic flexibility", easily switching between different

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Received: 5 July 2023; Revised: 29 November 2023; Accepted: 30 November 2023; Published: 27 December 2023
https://doi.org/10.54844/mtm.2023.0422

energy substrates to maintain ATP production.[8] The main fuels for resting platelets include glucose, fatty acids, glutamine, and acetate in the blood.[9,10] Glutamine also serves as a substrate for mitochondrial respiration, although platelets rely less on it as a metabolic source of fuel.[10]

GLUCOSE METABOLISM IN ACTIVATED PLATELETS AND THROMBOSIS

Glucose is a rich and easily accessible nutritional source for platelets and is an important energy source regardless of whether platelets are at rest or activated. The transition of platelets from a resting state to an activated state promotes rapid uptake of exogenous glucose, which is closely related to the activity of the main glucose transporter protein, glucose transporter 3 (GLUT3) expressed by the cells.[11,12] An absence of GLUT3 can fundamentally change platelet function. [13,14] Most of the glucose taken up by platelets is used to produce energy through glycolysis. In contrast, the pentose phosphate pathway has only minor involvement in the total flux of glucose. Usually, this behavior of platelets activated by thrombin is described as the "glycolytic phenotype".[10] Notably, metabolic rearrangement in stimulated platelets is primarily biased towards aerobic glycolysis rather than mitochondrial respiration.[11,15] Although glycolysis has low efficiency for producing ATP, its fast production rate can respond to rapid changes in energy demand during platelet activation. Glycolysis and oxidative phosphorylation therefore compensate mutually to fulfill the bioenergetic requirements of activated platelets. The use of an inhibitor of mitochondrial respiration by itself has no impact on the platelet activation response. However, there is evidence that a combination of inhibitors of glycolysis and mitochondrial respiration or the small molecule water-soluble carbon monoxide-releasing molecule-A1 (CORM-A1), which simultaneously impedes glycolysis and mitochondrial respiration, results in inhibition of platelet aggregation.[16–18] However, interestingly mitochondrial respiration has been shown to be unable to completely compensate for the inhibition of glycolysis, while the use of the glycolysis inhibitors, dichloroacetate (DCA), diarylsulfonamide-58 (DASA), and selective pyruvate kinase M2 activator (ML265) alone inhibit aggregation associated with platelet activation, further emphasizing the important contribution of glycolysis to platelet activity.[11,19,20] In addition, it has been reported that the absence of an external glucose supply does not affect the conversion of the platelet glycolytic phenotype, indicating that platelets also utilize endogenous glycogen reserves to meet their energy needs.[10]

Glucose metabolism relies heavily on the activity of two main enzymes, pyruvate dehydrogenase (PDH) and pyruvate kinase M2 (PKM2). The negative regulation of adenosine monophosphate-activated protein kinase (AMPK) and Src tyrosine kinase contributes to the transition of activated platelets to a glycolytic phenotype.[18] Knockout of PKM2 in mice was shown to severely impair the response of platelets to agonist stimulation and thrombus formation.[19] In addition, following platelet activation, pyruvate dehydrogenase kinase (PDK) phosphorylates PDH to inhibit its activity, thereby shifting pyruvate flux from oxidative phosphorylation to aerobic glycolysis. Knockout of PDK has also been demonstrated to impede agonist-stimulated platelet activity and diminish the formation of arterial thrombosis.[20,21] Activated platelets stimulate the pentose phosphate pathway, thereby promoting the synthesis of nicotinamide adenine dinucleotide phosphate (NADPH), which acts as a substrate for NADPH oxidase (NOX), resulting in generation of reactive oxygen species (ROS). The activation of integrin αIIbβ3 on the platelet surface, which is crucial for platelet aggregation, is induced by ROS.[22] Inhibitors of the pentose phosphate pathway can therefore inhibit thrombosis.[11]

FATTY ACID METABOLISM IN ACTIVATED PLATELETS AND THROMBOSIS

Preserved mitochondrial function under the glycolytic phenotype indicates the necessity of ATP for redox metabolism beyond glycolysis that ensures cell survival.[23–25] This corresponds with the observation that platelets metabolize nearly all glucose into lactate by aerobic glycolysis, as opposed to being metabolized by the mitochondrial tricarboxylic acid cycle.[9] This implies that a significant portion of ATP produced in the mitochondria is derived from the oxidation of fatty acids. In this regard, the inhibitors of fatty acid β-oxidation, etomoxir, oxfenicine, and trimetazidine have been shown in mice to mitigate the activation, aggregation, and granule secretion of platelets, thereby inhibiting arterial thrombus formation.[9] Taken together, these findings indicate that fatty acid β-oxidation maintains ATP levels in activated platelets.

In addition to mitochondrial fatty acid oxidation, cytoplasmic fatty acid synthesis also plays an important role in platelet function and thrombus formation. Acetyl-CoA carboxylase (ACC) is a key regulatory enzyme in the fatty acid synthesis pathway, with its activity regulated by inhibition of its phosphorylation by AMPK. Studies have shown that inhibiting ACC phosphorylation promotes arterial thrombus formation both *in vivo* and *in vitro*.[26] Arachidonic acid is a well-known key mediator of platelet activation, and under conditions of ACC inhibition, the synthesis of

phosphatidylethanolamine phospholipids necessary for the production of arachidonic acid is increased. This increase in arachidonic acid promotes cyclooxygenase-1 to produce thromboxane A2 (TxA2), which in turn facilitates the release of adenosine diphosphate from dense granules, thereby promoting thrombus formation.

AMINO ACID METABOLISM IN ACTIVATED PLATELETS AND THROMBOSIS

Amino acids are essential nutrients for platelets. However, there are only a small number of studies on the relationship between amino acid metabolism and platelet activation responses. Branched-chain amino acids (BCAAs) aid in platelet activation and increase thrombus formation. Of the three BCAAs, valine (Val) and isoleucine (Ile) may have a more important role than leucine (Leu) in the regulation of platelet activation.[27] Although Leu, Ile, and Val alone promote platelet activation, the impact of ketoisovalerate (KIV) and ketomethylvalerate (KMV) (metabolites of Val and Ile, respectively) on enhancement of platelet activation is more pronounced than the Leu metabolite ketoisocaproic acid (KIC). Furthermore, propionyl-CoA, a common metabolite of KIV and KMV, significantly enhances platelet activation, further suggesting that the Val and Ile metabolic pathways are the main routes for regulating platelet activation. Propionyl-CoA promotes protein propionylation. In this regard, propionylation of tropomodulin 3 is increased in BCAA-treated platelets, which is an important mechanism for BCAAs to promote platelet activation and thrombus formation. Moreover, sulfur-containing amino acids such as methionine and cysteine correlate negatively with platelet activity and are important regulators of thrombus formation.[28,29] This regulation may be related to protein oxidation, although the specific mechanism requires further research.

EFFECTS OF METABOLIC REGULATION ON PLATELETS

At present, some small molecules have been found to regulate platelet energy metabolism, thereby inhibiting thrombosis (Table 1). The impact of regulating glycolysis on platelet function remains a subject of ongoing debate. Neither 2-deoxyglucose, a glycolysis inhibitor, nor antimycin and oligomycin, mitochondrial respiration inhibitors, impact platelet activation responses. Nevertheless, the simultaneous use of these inhibitors obstructs platelet activation responses such as alteration in shape, aggregation, release of arachidonate, and secretion of granules.[16,30] Similarly, inhibiting the oxidation of fatty acids or glutamine decreases mitochondrial respiratory function without affecting the process of platelet aggregation induced by thrombine.[15] Notably, CORM-A1 effectively prevents platelet aggregation by simultaneously inhibiting both glycolysis and mitochondrial respiration.[18] These findings indicate that glycolysis and mitochondrial respiration mutually substitute to fulfill the energy demands necessary for platelet activation.

However, recent research has revealed a paradox in that mitochondrial respiration does not fully compensate for glycolysis and 2-deoxyglucose inhibits platelet aggregation.[31] In addition, DCA and dehydroepiandrosterone sulphate (DHEAS) (*i.e.*, an inhibitor of PDK), as well as DASA and ML265 (*i.e.*, activators of PKM2), inhibit platelet responses by regulating glycolysis, emphasizing the important role of glycolysis in platelet responses.[11,19,20] However, in addition to inhibiting glycolysis, the antiplatelet effects of these small molecules may be mediated by a variety of other mechanisms. DASA has the ability to prevent the production of ROS by blocking NOX activity, which is an important medium for platelet activation.[11] DCA also inhibits TxA2 production and tyrosine phosphorylation of spleen tyrosine kinase (Syk) and phosphatidylinositol-specific phospholipase Cγ2 (PLCγ2) in platelet activation,[20] while ML265 induces impairment of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling axis.[19] In addition, inhibition of glycolysis by knocking out glucose transporter 1 (GLUT1) and GLUT3,[14,32] or the glycolytic enzymes PKM2[19] and PDK,[21] severely impairs agonist-induced platelet responses and thrombosis.

ATP plays a crucial role in platelet granule secretion and thrombosis, with most mitochondrial ATP being derived from the oxidation of fatty acids. Etomoxir and oxfenicine are known to inhibit carnitine palmitoyltransferase-1 (CPT1), while trimetazidine has been shown to inhibit the enzyme, long-chain 3-ketoacyl-CoA thiolase (LCKAT), thereby causing significant impairment of fatty acid oxidation and contributing to depletion of ATP levels.[9] In this way, trimetazidine and oxfenicine effectively suppress the aggregation of platelets and granule secretion. Moreover, mice administered either etomoxir, trimetazidine, or oxfenicine exhibit a protective effect against thrombosis. In summary, β-oxidation of fatty acids sustains ATP levels in activated platelets and plays a critical role in thrombosis.

CONCLUSION

Activated platelets undergo metabolic changes that are crucial for thrombus formation. In both physiological or pathological conditions, platelets display metabolic flexibility and dependency in energy substrate selection. Enhancing our understanding of platelet metabolism and its regulation may therefore aid in the development of new therapeutic targets for the treatment of arterial

Table 1: Small molecule regulator of platelet metabolism

Small molecule	Modulation of metabolic pathways
2-deoxyglucose	Inhibited glycolysis
Antimycin, oligomycin	Impaired mitochondrial respiration
CORM-A1	Simultaneously inhibited glycolysis and mitochondrial respiration
DCA, DHEAS	Downregulated glycolysis by inhibiting PDK, and inhitited TxA2 production and tyrosine phosphorylation of Syk and PLCγ2
DASA-58, ML265	Suppressed glycolysis by activating PKM2, restrained NOX activity and ROS production, and imparied PI3K/Akt signaling axis
Etomoxir, oxfenicine	Disrupted fatty acid oxidation by inhibiting CPT1
Trimetazidine	Disrupted fatty acid oxidation by inhibiting LCKAT

CORM-A1, carbon monoxide-releasing molecule-A1; DCA, dichloroacetate; DHEAS, dehydroepiandrosterone sulphate; DASA-58, diarylsulfonamide-58; PDK, pyruvate dehydrogenase kinase; TxA2, thromboxane A2; Syk, spleen tyrosine kinase; PLCγ2, phosphatidylinositol-specific phospholipase Cγ2; PKM2, pyruvate kinase M2; NOX, nicotinamide adenine dinucleotide phosphate oxidase; ROS, reactive oxygen species; PI3K/Akt, phosphatidylinositol 3-kinase/protein kinase B; CPT1, carnitine palmitoyltransferase-1; LCKAT, long-chain 3-ketoacyl-CoA thiolase.

thrombosis. At present, the treatment of thrombus by regulating platelet metabolism remains controversial. In this regard, platelet metabolism is highly flexible and therefore the effect of metabolic regulation on platelet function requires further comprehensive discussion.

DECLARATIONS

Author contributions

Fei M: Writing—Original draft, Writing—Review and Editing, Visualization, Supervision, Project administration. Guo YS: Conceptualization, Resources, Supervision, Project administration, Funding acquisition. Wang HQ, Lin N: Investigation, Writing—Original draft. All authors have read and approve the final version.

Source of funding

This work was supported by the National Natural Science Foundation of China General Program (No. 81873495, No. 82171569), Heart Failure Center Research Foundation of Fujian Provincial Hospital (supported by Fujian Provincial Department of Finance), National Key Clinical Specialty Construction Project of China (Cardiovascular Medicine 2021) and Cardiovascular Disease Clinical and Research Platform (National Regional Medical Center).

Ethical approval and informed consent

Not applicable.

Conflict of interest

The author has no conflicts of interest to declare.

Data sharing

No additional data.

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