Modulation of microRNAs by aspirin in cardiovascular disease

Modulation of microRNAs by aspirin in cardiovascular disease

Abstract:

Aspirin is the most widely prescribed drug in cardiovascular and cerebrovascular diseases for both primary and secondary prevention. The major mechanisms underlying its benefits are the inhibitory effects on platelet activation and on prostanoid biosynthesis induced by COX-1 and COX-2 inactivation. MicroRNAs (miRNAs) are newly proposed mediators of the effects of aspirin. In this review, we summarize the evidence on the links between miRNAs and aspirin use in relation to cardiovascular diseases. In addition, we discuss the studies suggesting a possible role for miRNAs as biomarkers of aspirin resistance, a condition during which atherothrombotic events occur despite aspirin and which affect a considerable proportion of patients with cardiovascular diseases.

Key words: Aspirin; MicroRNA; Thrombosis; Cardiovascular disease; aspirin resistance

Introduction

MicroRNAs (miRNAs) are a class of small non-coding RNAs that post-transcriptionally regulate gene expression by inhibition of mRNA translation or induction of mRNA degradation (Mirzaei et al., 2016). These molecules are involved in various physiological and pathological processes, examples being cardiovascular events (Barwari, Joshi, & Mayr, 2016; Mirzaei et al., 2018; Romaine, Tomaszewski, Condorelli, & Samani, 2015), cancer (Fathullahzadeh, Mirzaei, Honardoost, Sahebkar, & Salehi, 2016; Ghandadi & Sahebkar, 2016; Jansson & Lund, 2012; Momtazi et al., 2016; Moridikia, Mirzaei, Sahebkar, & Salimian, 2018; Peng & Croce, 2016), diabetes mellitus (Paseban, Butler, & Sahebkar, 2019), etc. Platelets are nuclear cellular

fragments that originates from megakaryocytopoiesis; despite the absence of genomic DNA and a nucleus, post-transcriptional gene regulation can still occur due to the presence of the necessary spliceosome factors (Lindsay & Edelstein, 2016; McManus & Freedman, 2015).

Cyclooxygenase (COX) has two distinct membrane-anchored functional isoenzymes in humans: COX-1 and COX-2. COX-1 is constitutively expressed in most normal tissues while COX-2 is highly induced by proinflammatory mediators. COX-1 is the predominant isoform in normal vessels with constitutive expression in the endothelium and irregular expression in the vascular smooth muscles. On the contrary, COX-2 is not expressed in the majority of normal endothelial or vascular smooth muscle cells while it could be rapidly induced with vessel trauma or inflammation (Sellers, Radi, & Khan, 2010) Aspirin is an analgesic and anti-inflammatory drug that works as an irreversible inhibitor of COX-1. COX-1 is the catalytic enzyme of arachidonic acid conversion to prostaglandins G_2, H_2 and subsequently to thromboxane A_2; it is largely found in platelets but is not restricted to that location (Warner, Nylander, & Whatling, 2011). Thromboxane A_2 acts as a vasoconstrictor, a proliferative factor for vascular smooth muscle cells and also a platelet aggregator. The COX-1 enzyme inhibition is irreversible and persists for the entire lifespan of the platelets (Smyth, 2010). Aspirin also inhibits COX-2, though to a lesser extent (Warner et al., 2011).

Besides the irreversible inhibition of COX-1, aspirin acts through other mechanisms in the prevention of cardiovascular disease, such as platelet inactivation by inhibition of P-selectin glycoprotein favoring leukocytes recruitment and rolling, and inhibition of platelet factors and fibrinogen which favour the development of thrombosis. Aspirin also prevents thrombin formation which is the convertor of fibrinogen to fibrin or influence the quality of fibrin within the thrombus. Increasing the rate of fibrinolysis is another mechanism of action of aspirin that is related to the acetylation of fibrinogen (Mekaj, Daci, & Mekaj, 2015). A pharmacokinetic and pharmacodynamic study of aspirin in 22 healthy volunteers showed almost complete inhibition of platelet function 20 minutes and 5 minutes after its administration in oral or intravenous form respectively (Nagelschmitz et al., 2014). There are few small and heterogenous studies investigating the impact of aspirin on the function of vessels and blood pressure in patients

with arterial hypertension which are not adequate for drawing reliable conclusions (Dzeshka, Shantsila, & Lip, 2016). Further studies are needed to address this issue.

Besides the anti-thrombotic, antipyretic, and analgesic properties of aspirin, a large number of human studies have provided convincing evidence for a considerable reduction in the risk of cancer with regular low-dose use of aspirin. The chemopreventive properties of aspirin could be attributed to its platelet modulating effect (Ornelas et al., 2017).

Besides the COX-dependent mechanisms, COX-independent ones are also involved, and among them miRNA have received attention in recent years. Therefore, in this review we summarize the data related to miRNAs modulation following aspirin administration as it relates to cardiovascular disease. We also look at the data that address miRNAs as potential diagnostic or even prognostic markers in aspirin resistance (Fig 1).

Aspirin, miRNA, and cardiovascular disease

In spite of decades of wide administration of low-dose aspirin for primary prevention of atherosclerotic cardiovascular disease, the results of three large randomized controlled primary prevention clinical trials, the ASCEND trial (15,480 participants with diabetes mellitus and no evident cardiovascular disease, with median follow up of 7.4 years) ("Effects of Aspirin for Primary Prevention in Persons with Diabetes Mellitus," 2018), the ARRIVE trial (A Study to Assess the Efficacy and Safety of Enteric-Coated Acetylsalicylic Acid in Patients at Moderate Risk of Cardiovascular Disease; 12,546 non-diabetic participants, with median follow up of 5 years) (Gaziano et al., 2018), and the ASPREE trial (Aspirin in Reducing Events in the Elderly study; 19,114 participant without known cardiovascular diseases, with median follow up of 4.7 years) (McNeil et al., 2018) revealed a lack of net benefit for aspirin since the elevated risk of bleeding with its use was much higher than the preventive role towards atherosclerotic cardiovascular diseases. These evidences led to the revision of the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease, with the following recommendations:

- Low-dose aspirin might be considered for primary prevention of atherosclerotic cardiovascular diseases (ASCVD) in selected higher-risk adults aged 40-70 years who are not at increased bleeding risk.
- Low-dose aspirin should not be administered on a routine basis for primary prevention of ASCVD among adults >70 years.
- Low-dose aspirin should not be administered for primary prevention among adults at any age who are at increased bleeding risk (Arnett DK, .2019).

In addition, the US Food and Drug Administration (FDA) does not recommend aspirin for the primary prevention of heart attacks and strokes for the general population, while its use should be considered limited to those individuals for whom the benefits outweigh the risks (Huang & Yang, 2015). Therefore, aspirin use is supported by strong evidence of overweighing benefits vs potential risks only in the secondary prevention of cardiovascular disease (Millard & Hernandez-Vila, 2018).

At present, very few studies are available about the potential role of miRNAs in determining/modulating the effects of aspirin in the prevention of cardiovascular diseases.
The most abundant among the 532 miRNAs that has been recognized in platelets of healthy human are the members of let-7 family (Sisodia & Bhatia, 2018).

There are three types of platelet secretory granules including α–granules, dense granules, and lysosomes. Among them, α–granules are the most abundant and necessary for the activity of platelets. Following the activation of platelets, α–granules fuse with the plasma membrane and release their content. Different functional roles, implicated in the pathogenesis of cardiovascular diseases have been identified for platelet α–granules, such as pro-coagulative and pro-inflammatory effects, and treatments specifically targeting their content release might be used to control these processes (Blair & Flaumenhaft, 2009). miR-21 may modulate proteins that regulate the release of α-granule from platelets; proteomics analysis of the platelet releasate (content?) showed that treatment with antagonists of miR-21 affects the release of α–granule proteins, such as TGF-β1, von Willebrand factor, and fibronectin (Barwari et al., 2018).

Considering the high clinical importance of aspirin in the management of coronary artery disease, we are going to discuss the role of miRNA in diagnostic tests of aspirin resistance along with its role in proposed mechanisms of aspirin resistance.

1) Platelet reactivity/ASA resistance

Aspirin resistance is defined as aspirin inability in the reduction of thromboxane A2 production by platelets which causes impaired suppression of platelet activation and aggregation and has been associated with an increased cardiovascular risk. The following mechanisms have been implicated in aspirin resistance: inadequate aspirin dosage or patient compliance, aspirin interactions with drugs like nonsteroidal anti-inflammatory drugs (NSAIDs), genetic polymorphisms, upregulation of thromboxane biosynthesis in non-platelet sources, and increased turnover of platelets. Therefore, different strategies should be used to overcome aspirin resistance based on the type of cause(s). Developing reliable tests is necessary to investigate the potential mechanisms of action and to investigate the efficacy of treatments (Hankey & Eikelboom, 2006).

Aspirin resistance is experienced in approximately one fourth of cardiovascular patients. (Du, Lin, & Wang, 2016). Variability of platelet reactivity among cardiovascular patients treated with antiplatelet drugs such as aspirin is a matter of concern and has been shown to be not only related to inter-individual genetic variations but also to epigenetic factors such as miRNAs. miR-135a-5p and miR-204-5p are two candidate miRNAs that are correlated with platelet reactivity and synergistically effect a group of candidate genes (THBS1, CDC42, CORO1C, SPTBN1, TPM3, GTPBP2, and MAPRE2); these genes were identified via a network biology approach using proteomic and transcriptomic data from two groups of patients, either with extremely high or extremely low platelet reactivity (Zufferey et al., 2016). Another group measured sustained platelet aggregation following incubation with indomethacin, a drug which mimics aspirin effect; they reported, a relationship between lower expression of miR-19b-1-5p and aspirin insensitivity and proposed miR-19b-1-5p as a suitable marker for aspirin insensitivity (Kok et al., 2016).

An association of circulating miR-223 and platelet reactivity has been reported in patients suffering from coronary artery disease who undergo dual antiplatelet therapy with aspirin and clopidogrel (Chyrchel et al., 2015) This was a proof-of-concept study for identification of potential platelet miRNAs that could be substitute markers to determine the efficiency of antiplatelet therapy. MiR-126, miR-197, miR-223, miR-24, and miR-21 were discovered by microarray screening as the most highly expressed miRNAs in platelets and platelet microparticles. Among them, a low circulating level of miR-223 was shown to be an independent predictor of poor prognosis associated conditions, such as myocardial infarction, according to the population based study of Bruneck (Zampetaki et al., 2012), and type 2 diabetes (Chyrchel et al., 2015; Duan et al., 2014).

Comparing plasma circulating level of miR-223 quantified by real time PCR between normal-responders and low-responders to antiplatelet therapy with clopidogel and aspirin with troponin-negative non-ST elevation acute coronary syndrome, a decreased level of miR-223, but not other factors (including CYP2C19*2/*3 loss-of-function genotypes, use of calcium channel blockers/proton-pump inhibitors, age, diabetes and smoking) resulted an independent predictor for responsiveness to antiplatelet therapies (Zhang et al., 2014).

There is an association between overexpression of multidrug resistance protein-4 (MRP4), an ATP binding cassette membrane transporter with active role in extrusion of pharmacological and physiological molecules, and reduction in post by-pass efficacy of aspirin. Using Real time PCR, flow cytometry, and western blotting techniques, a higher expression of this transporter along with lower levels of MiR-26b were observed in patients who had a chronic history of aspirin administration in comparison with the control group. In addition, a significant reduction in MPR4 level was observed following the transfection of platelets with MiR-26b so it can be one of miRs that play a role in aspirin resistance (La Rosa et al., 2018).

Platelet resistance could potentially be identified with a combination of circulating levels of miR-92a and the platelet distribution width (PDW) assay. The arachidonic acid stimulated aggregation test Multiplate analyzer (ASPItest) has been widely used to identify aspirin resistance and aspirin responders. The cut-off values for discrimination of these two groups are

≥30 U in the ASPItest, N 11.8 fL in the PDW test, and a relative expression level of 4.5 for miR-92a. A PDW/miR-92a-score using these cut-off values could successfully detect aspirin resistance with the positive and negative predictive values of 88.9% and 95.1%, respectively. Routine laboratory tests in current use for evaluating platelet function suffer from some limitations, including inter-and intra-individual variability, possible inaccuracy due to *in vitro* and *in vivo* differences and the need to perform the test within a brief window of 30 to 120 minutes post sampling. Therefore, this new method could potentially represent an advance for discriminating patients who would benefit from platelet inhibition with aspirin from those who would not (H. G. Binderup, Houlind, Madsen, & Brasen, 2016). In a validation cohort, both PDW and plasma levels of miR-92a were confirmed to be significantly higher in patients who are aspirin resistant in comparison to responding individuals; however, researchers failed to validate the newly developed score was not successful in confirming the high sensitivity of the before mentioned pilot study so they used new identified cut-off values based on validation cohort to obtain a specificity of 75% and a sensitivity of 54.9% (H. Binderup, Houlind, Lohman Brasen, & Madsen, 2018).

Two main issues are still waiting to be addressed in validation clinical studies. First, we don't know how to manage the patients who are poor responders to aspirin, and then we do not know if the laboratory monitoring of aspirin therapy is really cost-effective (Cattaneo, 2007).

2) Endothelial and Vascular Smooth muscle cells

Aspirin is a drug used both for primary prevention of cerebrovascular and cardiovascular disease (Paseban, Mohebbati, Niazmand, Sathyapalan, & Sahebkar, 2019) (Hennekens & Dalen, 2014) and also for secondary prevention of recurrent ischemic vascular events (Boonyawat & Crowther, 2015). Although these effects are mainly mediated through COX inhibition, miRs are also involved in both aspirin's cardiovascular benefits and aspirin resistance (Table 1).

Abnormal proliferation of vascular smooth muscle cells is one of the pathological features in atherosclerosis, which underlies, among other, ischemic stroke (Doran, Meller, & McNamara, 2008; Orr, Hastings, Blackman, & Wamhoff, 2010).The anti-proliferative and anti-inflammatory

effects of aspirin on vascular smooth muscle cells are mediated through inhibition of CD40 mRNA translation by miR-145. This inhibitory effect improves the stability of atherosclerotic plaques By comparing pre and post aspirin treatment level of miR-145 in 46 ischemic stroke patients, it was revealed that ten days aspirin treatment elevated the level of this miR in peripheral blood mononuclear cells. In addition, this increase in miR-145 and decrease in CD40 expression was more evident in atherosclerotic plaques of aspirin-treated ischemic stroke patients compared to those untreated. In vitro studies reported in vascular smooth muscle cells a significant decrease in IL-6 levels and a significant suppression of cells proliferation and CD40 mRNA expression following aspirin treatment. These effects were reversed when a miR-145 inhibitor was used to suppress the expression of this miRNA. In this study, miR-145 was measured by Real-time PCR after transfection with either miR-145 inhibitor (50, 100, 200 nmol/L) or the miR-145 inhibitor control for 24 h, and the stem loop primers of miR-145 was used to amplify miR-145 in the peripheral blood mononuclear cells from IS patients. Furthermore, treatment of ischemic stroke patients with aspirin for 10 days significantly increased the expression of miR-145 in peripheral blood mononuclear cells (Guo et al., 2016).

Anti-platelet therapy reduces plasma levels of platelet-related miRNAs, including miR-126 and miR-223 (Willeit et al., 2013). The link between miR-126 and atherogenesis has already been recognized (Weber & Noels, 2011). MiR-126, highly expressed in endothelial cells and platelets and suppressed in aspirin responders, is a promising prognostic biomarker of vascular damage and endothelial dysfunction. miR-223 has been used to categorize patients as 'responder' and 'non-responder' to the clopidogrel which is a P2Y12 inhibitor (R. Shi et al., 2013). In an interesting study, the correlation of plasma miRNA levels with platelet function was studied in 125 patients who had a past 30 days history of acute coronary syndrome [ref]. Among the identified miRNAs with next-generation sequencing of small RNAs in plasma, miR-126 and miR-223 showed the greatest platelet dependency [ref]. Platelet aggregation was reduced in mice with inhibited miR-126 and this miR affects both directly and indirectly the expression of ADAM9, a predicted and experimentally confirmed target of miR-126 that acts as an accelerator of the adhesion of platelets to collagen (Cominetti et al., 2009) in addition, it is a protease of the ADAM family so by cleaving membrane proteins and also the expression of P2Y12 receptor

which plays such an important role in platelet reactivity it may alter the platelet response, these targets could explain differences in platelets reactivity (Kaudewitz et al., 2016).

However, there is concern that antiplatelet drugs like aspirin and lipid-lowering medications like statins may affect the profile of circulating miRNAs (Mohajeri et al., 2018) (Paseban, Butler, et al., 2019). For example, aspirin has been shown to suppress the release of miR-126 following ex vivo activation of platelets (Cavarretta, Chiariello, & Condorelli, 2013).

When considering the use of miR-126 as a proposed biomarker for endothelial dysfunction in type 2 diabetes (DM2) and coronary artery disease (CAD), the fact that this miRNA is abundant not only in endothelial cells but also in platelets must be taken into account. Both *in vitro* and *in vivo* activation of platelets, with the resultant confounding effect on the plasma level of miR-126, could be impacted by aspirin administration, especially in pathophysiological conditions associated with platelet activation like DM2 (Willeit et al., 2013). In a study conducted to show how aspirin could affect the miR-126 plasma pool of DM2 patients through modulation of platelets originated compartment, following the administration of aspirin both platelet inhibition and reduction in platelet derived miR-126 was observed and in case of using miR-126 as a biomarker we should consider the effect of aspirin administration on the level of this miR as platelets are a major source in this case (de Boer et al., 2013). In order to determine the possible association between miRNA levels and clinical outcome in patients with acute CAD, large cohort studies with prolonged follow-up are necessary.

Resolvins and Lipoxins

Lipoxins and resolvins are anti-inflammatory and inflammatory-resolving lipid mediators, respectively. They are among the first mediators identified that actively promote the resolution of inflammation (Serhan, Chiang, & Van Dyke, 2008).

Lipoxins are lipoxygenase interaction products that are synthesized from arachidonic acid by three major routes. In the first one, taking place in platelets, 12-Lo lipoxygenase converts leukotriene A4 to lipoxins (Serhan & Sheppard, 1990); in the second route, in neutrophils, erythrocytes and reticulocytes, 5-LO lipoxygenase and 15-LO lipoxygenase, respectively, act in

series to convert arachidonic acid to lipoxin A and lipoxin B.3; the third route is aspirin dependent and generates aspirin-triggered lipoxin (ATL) and 15 epi-lipoxin B4 (Chiang, Bermudez, Ridker, Hurwitz, & Serhan, 2004). It has been proved that local anti-inflammatory actions of low-dose aspirin in healthy individuals is mediated through ALT generation (Chiang et al., 2004; Morris et al., 2009).

Acetylation of COX-2 following aspirin administration leads to epi-lipoxins formation and also aspirin could amplify epi-lipoxins formation by nitrosylation of statin-induced COX-2 (Planaguma et al., 2010). Resolvins are specialized lipid mediators which promote the resolution of acute inflammation and could be divided in two classes, the E-series resolvins (RvE1, RvE2, and RvE3) which synthetized from eicosapentaenoic acid and the D series resolvins (RvD1–RvD6) derived from docosaesaenoic acid. RvD1 is the regulator of miRNAs and their target genes associated with resolution of acute inflammation (Recchiuti, Krishnamoorthy, Fredman, Chiang, & Serhan, 2011). It has been shown that RvD1 selectively interacts with receptors ALX/FPR2 and GPR32; the administration of RvD1 in ALX/FPR2 transgenic mice significantly up-regulated miR-208a which then downregulated PDCD4, a proinflammatory regulatory protein acting both as an IL-10 inhibitor and a promoter of the NF-κB pathway (Recchiuti et al., 2011) along with upregulation of miR-219 targets 5-lipoxygenase and regulates leukotriene B4 production (Recchiuti et al., 2011). These two miRs are both endogenously expressed in resident peritoneal cells. Overexpression of miR-208a in human macrophages comes with IL-10 upregulation (Krishnamoorthy, Recchiuti, Chiang, Fredman, & Serhan, 2012). The role of miRNAs in modulating the cardiovascular effects of aspirin through resolvins or lipoxins warrants to be studied by further research.

3) ADP receptor antagonists

Ticlopidine, clopidogrel, and prasugrel are antagonists of the P2Y12 platelet adenosine diphosphate (ADP) receptor. ADP P2Y12 is one of the genes regulated by miR-223, a miRNA abundant in platelets, and also a key target of antiplatelet therapy. The circulating level of this miR has reported to be inversely associated with major cardiovascular events in patients with CAD receiving antiplatelet treatment (Shi et al., 2015). Profile of microRNAs that are released

following the stimulation of washed platelets from healthy subjects with agonists specific for the collagen (Glycoprotein VI (GPVI)), thrombin (PAR1/PAR4), or ADP (P2Y1/P2Y12) using TaqMan microRNA microarray cards showed that following the activation of platelets a 46 members core of miRNAs was observed among them Mir-223-3p with role in myeloid linage development and anti-inflammatory effects was the most abundant. The role of ADP (P2Y1/P2Y12) in release of microRNAs was very important (Ambrose, Alsahli, Kurmani, & Goodall, 2018).

Significant inter-individual variability in pharmacokinetics exists among patients with a past myocardial infarction or stroke who receive clopidogrel, one of the most commonly used drugs for the secondary prevention of atherothrombotic events. About 1/3 of these patients receive no benefit from Clopidogrel; genetic variability in intestinal drug efflux through permeability glycoproteins (P-gp) and in metabolizing enzymes such as the cytochrome P450 (CYP) which converts inactive pro-drug to the active thiol metabolite are possible underlying causes. Aspirin, commonly co-administered with clopidogrel, decreases the bioavailability of clopidogrel by decreasing oral absorption and inducing intestinal permeability P-gp expression. The effect of aspirin administration on pharmacokinetic of clopidogrel was investigated in 18 healthy volunteers with CYP2C19 and PON1 genotypes of cytochrome P450, an important metabolizing enzyme affecting the bioactivation of absorbed clopidogrel [ref]. An increase (up to 7.67-fold) in the expression of miR-27a was found… (Oh et al., 2014). MiR-27a is known to upregulate the expression of P-gp protein by inhibiting transcriptional factors such as phospholipase C/Raf/mitogen-activated protein kinase pathway or the C-terminal-binding protein 1, all suppressing P-gp expression (Zhu et al., 2008). After coadministration with low dose of aspirin (2 and 4 weeks of once-daily 100-mg aspirin administration), the antithrombotic efficacy of clopidogrel was not decreased (Oh et al., 2014). Aspirin significantly increased the level of plasma miR-27a, which peaked at 1 week after once-daily administration (Zhu et al., 2008).

Among six miRNAs which were screened by high-throughput illumina sequencing in the plasma of patients with CAD undergoing coronary angiography and antiplatelet therapy with clopidogrel and aspirin, a high level of miR-142 resulted an independent risk factor of major

adverse cardiovascular events (Tang et al., 2019). This result was subsequently validated in 1230 patients with CAD, thus suggesting miR-142 as a potential predictive marker in patients with pre-existent cardiovascular diseases (Tang et al., 2019).

Future directions

By clarifying the modulating effect of aspirin on miRNAs in the primary and secondary prevention of cardiovascular diseases, a better comprehension of the possible contributors to success or failure to aspirin treatment might be recognized and used to identify patients who benefit greatly from this therapy. Another topic worth to be studied is the possible use of miRNAs as a fast, reliable method of identification of patients with aspirin resistance.

Conclusion

MiRNAs play an important role in platelet function. Platelets make a substantial contribution to the circulating miRNA pool. Some miRNAs have been identified as antiplatelet responsive. However, the potential confounding effects of the antiplatelet therapy should be considered when interpreting the case-control studies relating to circulating miRNA in cardiovascular disease. Furthermore, miRNAs could be used as *in vivo* biomarkers of assessing platelet responses rather than the current *ex vivo* ones. At present, a few studies are available on miRNAs and aspirin and further investigations are required.

References

Ambrose AR, Alsahli MA, Kurmani SA, Goodall AH. Comparison of the release of microRNAs and extracellular vesicles from platelets in response to different agonists. Platelets. 2018;29:446-454. doi:10.1080/09537104.2017.1332366

Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Golberger ZD, Hahn EJ, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2019; in press. doi:10.1016/j.jacc.2019.03.009

ASCEND study collaborative group. Effects of Aspirin for Primary Prevention in Persons with Diabetes Mellitus. New Engl J Med. 2018;379:1529-1539. doi:10.1056/NEJMoa1804988

Barwari T, Eminaga S, Mayr U, Lu, R, Armstrong PC, Chan MV, et al. (2018). Inhibition of profibrotic microRNA-21 affects platelets and their releasate. JCI Insight. 2018;3:123335. doi:10.1172/jci.insight.123335

Barwari T, Joshi A, Mayr M. MicroRNAs in Cardiovascular Disease. Journal of the American College of Cardiology. 2016;68:2577-2584. doi:10.1016/j.jacc.2016.09.945

Binderup H, Houlind K, Lohman Brasen C, Madsen J. (2018). Identification of aspirin resistance using a PDW-miR92a-score: Validation in an intermittent claudication cohort (Vol. 64). TO BE CORRECTED

Binderup HG, Houlind K, Madsen JS, Brasen CL. Aspirin resistance may be identified by miR-92a in plasma combined with platelet distribution width. Clin Biochem. 2016;49:1167-1172. doi:10.1016/j.clinbiochem.2016.04.017

Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. Blood reviews. 2009;23:177-189. doi:10.1016/j.blre.2009.04.001

Boonyawat K, Crowther MA. Aspirin in secondary prevention of recurrent venous thromboembolism. J Thromb Thrombolysis. 2015;39:392-394. doi:10.1007/s11239-015-1196-4

Cattaneo M. Laboratory detection of 'aspirin resistance': what test should we use (if any)? Eur Heart J. 2007;28:1673-1675. doi:10.1093/eurheartj/ehm232

Cavarretta E, Chiariello GA, Condorelli G. Platelets, endothelium, and circulating microRNA-126 as a prognostic biomarker in cardiovascular diseases: per aspirin ad astra. Eur Heart J. 2013; 34:3400-3402. doi:10.1093/eurheartj/eht032

Chiang N, Bermudez EA, Ridker PM, Hurwitz S, Serhan CN. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. Proc Natl Acad Sci U S A. 2004;101:15178-15183. doi:10.1073/pnas.0405445101

Chyrchel B, Toton-Zuranska J, Kruszelnicka O, Chyrchel M, Mielecki W, Kolton-Wroz M, et al. Association of plasma miR-223 and platelet reactivity in patients with coronary artery disease on dual antiplatelet therapy: A preliminary report. Platelets. 2015;26:593-597. doi:10.3109/09537104.2014.974527

Cominetti MR, Martin AC, Ribeiro JU, Djaafri I, Fauvel-Lafeve F, Crepin M, et al. Inhibition of platelets and tumor cell adhesion by the disintegrin domain of human ADAM9 to collagen I under dynamic flow conditions. Biochimie. 2009;91:1045-1052. doi:10.1016/j.biochi.2009.05.012

de Boer HC, van Solingen C, Prins J, Duijs JM, Huisman MV, Rabelink TJ, et al. Aspirin treatment hampers the use of plasma microRNA-126 as a biomarker for the progression of vascular disease. Eur Heart J. 2013;34:3451-3457. doi:10.1093/eurheartj/eht007

Doran AC, Meller N, McNamara CA. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. Arterioscler Thromb Vasc Biol. 2008;28:812-819. doi:10.1161/atvbaha.107.159327

Du G, Lin Q, Wang, J. A brief review on the mechanisms of aspirin resistance. Int J Cardiol. 2016;220:21-26. doi:10.1016/j.ijcard.2016.06.104

Duan X, Zhan Q, Song B, Zeng S, Zhou J, Long Y, et al. Detection of platelet microRNA expression in patients with diabetes mellitus with or without ischemic stroke. J Diab Compl. 2014;28:705-710. doi:10.1016/j.jdiacomp.2014.04.012

Dzeshka MS, Shantsila A, Lip GYH. Effects of Aspirin on Endothelial Function and Hypertension. Curr Hypert Rep. 2016;18:83-83. doi:10.1007/s11906-016-0688-8

Fathullahzadeh S, Mirzaei H, Honardoost MA, Sahebkar A Salehi M. Circulating microRNA-192 as a diagnostic biomarker in human chronic lymphocytic leukemia. Cancer Gene Ther. 2016;23:327-332. doi:10.1038/cgt.2016.34

Gaziano JM, Brotons C, Coppolecchia R, Cricelli C, Darius H, Gorelick PB, et al. Use of aspirin to reduce risk of initial vascular events in patients at moderate risk of cardiovascular disease (ARRIVE): a randomised, double-blind, placebo-controlled trial. Lancet. 2018;392:1036-1046. doi:10.1016/S0140-6736(18)31924-X

Ghandadi M, Sahebkar A. Microrna-34a and its target genes: Key factors in cancer multidrug resistance. Curr Pharm Des. 2016;22:933-939. doi:10.2174/1381612822666151209153729

Guo X, Yu L, Chen M, Wu T, Peng X, Guo R, et al. miR-145 mediated the role of aspirin in resisting VSMCs proliferation and anti-inflammation through CD40. J Transl Med. 2016;14:211. doi:10.1186/s12967-016-0961-2

Hankey GJ, Eikelboom JW. Aspirin resistance. Lancet. 2006;367:606-617. doi:10.1016/s0140-6736(06)68040-9

Hennekens CH, Dalen JE. Aspirin in the primary prevention of cardiovascular disease: Current knowledge and future research needs. Trends Cardiovasc Med. 2014;24:360-366. doi:10.1016/j.tcm.2014.08.006

Huang QY, Yang RQ. Should aspirin be used for the primary prevention of cardiovascular disease in the general population? Med Princ Pract. 2015;24:198-198. doi:10.1159/000368361

Jansson MD, Lund AH. MicroRNA and cancer. Mol Oncol. 2012;6:590-610. doi:10.1016/j.molonc.2012.09.006

Kaudewitz D, Skroblin P, Bender LH, Barwari T, Willei, P, Pechlaner R, et al. Association of MicroRNAs and YRNAs With Platelet Function. Circul Res. 2016;118:420-432. doi:10.1161/CIRCRESAHA.114.305663

Kok MG, Mandolini C, Moerland PD, de Ronde MW, Sondermeijer BM, Halliani A, et al. Low miR-19b-1-5p expression in isolated platelets after aspirin use is related to aspirin insensitivity. Int J Cardiol. 2016;203:262-263. doi:10.1016/j.ijcard.2015.10.098

Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN. Resolvin D1 receptor stereoselectivity and regulation of inflammation and proresolving microRNAs. Am J Pathol. 2012;180:2018-2027. doi:10.1016/j.ajpath.2012.01.028

La Rosa G, Biasucci LM, Mandolini C, Massimi I, Copponi G, Pulcinelli FM,et al. Platelet miRNA-26b down-regulates multidrug resistance protein 4 in patients on chronic aspirin treatment. J Cardiovasc Med (Hagerstown). 2018;19:611-613. doi:10.2459/jcm.0000000000000691

Lindsay CR, Edelstein LC. MicroRNAs in Platelet Physiology and Function. Semin Thromb Hemost. 2016; 42:215-222. doi:10.1055/s-0035-1570077

McManus DD, Freedman JE. MicroRNAs in platelet function and cardiovascular disease. Nat Rev Cardiol. 2015;12:711. doi:10.1038/nrcardio.2015.101

McNeil JJ, Wolfe R, Woods RL, Tonkin AM, Donnan GA, Nelson MR, et al. Effect of Aspirin on Cardiovascular Events and Bleeding in the Healthy Elderly. New Engl J Med. 2018:379:1509-1518. doi:10.1056/NEJMoa1805819

Mekaj YH, Daci FT, Mekaj AY. New insights into the mechanisms of action of aspirin and its use in the prevention and treatment of arterial and venous thromboembolism. Ther Clin Risk Manag. 2015;11:1449-1456. doi:10.2147/tcrm.s92222

Millard MA, Hernandez-Vila EA. What Do the Guidelines Really Say About Aspirin? Texas Heart Inst J. 2018;45:228-230. doi:10.14503/THIJ-18-6673

Mirzaei H, Gholamin S, Shahidsales S, Sahebkar A, Jaafari MR, Mirzaei HR, et al. MicroRNAs as potential diagnostic and prognostic biomarkers in melanoma. Eur J Cancer. 2016;53:25-32. doi:10.1016/j.ejca.2015.10.009

Mirzaei H, Momeni F, Saadatpour L, Sahebkar A, Goodarzi M, Masoudifar A, et al. MicroRNA: Relevance to stroke diagnosis, prognosis, and therapy. J Cell Physiol. 2018;233:856-865. doi:10.1002/jcp.25787

Mohajeri M, Banach M, Atkin SL, Butler AE, Ruscica M, Watts GF, et al. MicroRNAs: Novel Molecular Targets and Response Modulators of Statin Therapy. Trends Pharmacol Sci. 2018;39:967-981. doi:10.1016/j.tips.2018.09.005

Momtazi AA, Shahabipour F, Khatibi S, Johnston TP, Pirro M, Sahebkar A. Curcumin as a MicroRNA Regulator in Cancer: A Review. Rev Physiol Biochem Pharmacol. 2016;171:1-38. doi:10.1007/112_2016_3

Moridikia A, Mirzaei H, Sahebkar A, Salimian J. MicroRNAs: Potential candidates for diagnosis and treatment of colorectal cancer. J Cell Physiol. 2018;233:901-913. doi:10.1002/jcp.25801

Morris T, Stables M, Hobbs A, de Souza P, Colville-Nash P, Warner T, et al. Effects of low-dose aspirin on acute inflammatory responses in humans. J Immunol. 2009;183:2089-2096. doi:10.4049/jimmunol.0900477

Nagelschmitz J, Blunck M, Kraetzschmar J, Ludwig M, Wensing G, Hohlfeld, T. Pharmacokinetics and pharmacodynamics of acetylsalicylic acid after intravenous and oral administration to healthy volunteers. Clin Pharmacol. 2014;6:51-59. doi:10.2147/CPAA.S47895

Oh J, Shin D, Lim KS, Lee S, Jung KH, Chu K, et al. Aspirin decreases systemic exposure to clopidogrel through modulation of P-glycoprotein but does not alter its antithrombotic activity. Clin Pharmacol Ther. 2014;95:608-616. doi:10.1038/clpt.2014.49

Ornelas A, Zacharias-Millward N, Menter DG, Davis JS, Lichtenberger L, Hawke D, et al. Beyond COX-1: the effects of aspirin on platelet biology and potential mechanisms of chemoprevention. Cancer Metast Rev 2017;36:289-303. doi:10.1007/s10555-017-9675-z

Orr AW, Hastings NE, Blackman BR, Wamhoff BR. Complex regulation and function of the inflammatory smooth muscle cell phenotype in atherosclerosis. J Vasc Res. 2010;47:168-180. doi:10.1159/000250095

Paseban M, Butler AE, Sahebkar A. Mechanisms of statin-induced new-onset diabetes. J Cell Physiol. 2019;234:12551-12561. doi: 10.1002/jcp.28123

Paseban M, Mohebbati R, Niazmand S, Sathyapalan T, Sahebkar A. Comparison of the Neuroprotective Effects of Aspirin, Atorvastatin, Captopril and Metformin in Diabetes Mellitus. Biomolecules. 2019:9:118. doi: 10.3390/biom9040118

Peng Y, Croce CM. The role of MicroRNAs in human cancer. Sign Transd Targ Ther. 2016;1:15004. doi:10.1038/sigtrans.2015.4

Planaguma A, Pfeffer MA, Rubin G, Croze R, Uddin M, Serhan CN, et al. Lovastatin decreases acute mucosal inflammation via 15-epi-lipoxin A4. Mucosal Immunol. 2010;3:270-279. doi:10.1038/mi.2009.141

Recchiuti A, Krishnamoorthy S, Fredman G, Chiang N, Serhan CN. MicroRNAs in resolution of acute inflammation: identification of novel resolvin D1-miRNA circuits. Faseb J. 2011;25:544-560. doi:10.1096/fj.10-169599

Romaine SPR, Tomaszewski M, Condorelli G, Samani NJ. MicroRNAs in cardiovascular disease: an introduction for clinicians. Heart. 2015;101:921.

~~Sellers, R. S., Radi, Z. A., & Khan, N. K. (2010). Pathophysiology of cyclooxygenases in cardiovascular homeostasis. Vet Pathol, 47(4), 601-613. doi:10.1177/0300985810364389~~

Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008;8:349-361. doi:10.1038/nri2294

Serhan CN, Sheppard KA. Lipoxin formation during human neutrophil-platelet interactions. Evidence for the transformation of leukotriene A4 by platelet 12-lipoxygenase in vitro. J Clin Invest. 1990;85:772-780. doi:10.1172/jci114503

Shi R, Ge L, Zhou X, Ji WJ, Lu RY, Zhang YY, et al. Decreased platelet miR-223 expression is associated with high on-clopidogrel platelet reactivity. Thromb Res. 2013;131:508-513. doi:10.1016/j.thromres.2013.02.015

Shi R, Zhou X, Ji WJ, Zhang YY, Ma YQ, Zhang JQ, et al. The Emerging Role of miR-223 in Platelet Reactivity: Implications in Antiplatelet Therapy. BioMed Res Internat. 2015;981841-981841. doi:10.1155/2015/981841

Sisodia P, Bhatia R. Aspirin Resistance and Stroke. J Stroke Med. 2018;1:19-27. doi.org/10.1177/2516608518777017

476 Smyth EM. Thromboxane and the thromboxane receptor in cardiovascular disease. Clin Lipidol.
477 2010;5:209-219. doi:10.2217/clp.10.11
478 Tang QJ, Lei HP, Wu H, Chen JY, Deng CY, Sheng WS, et al. Plasma miR-142 predicts major adverse
479 cardiovascular events as an intermediate biomarker of dual antiplatelet therapy. Acta
480 Pharmacol Sin. 2019;40:208-215. doi:10.1038/s41401-018-0041-7
481 Warner TD, Nylander S, Whatling C. Anti-platelet therapy: cyclo-oxygenase inhibition and the use of
482 aspirin with particular regard to dual anti-platelet therapy. Br J Clin Pharmacol. 2011;72:619-
483 633. doi:10.1111/j.1365-2125.2011.03943.x
484 Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. Nat Med. 2011;
485 17:1410-1422. doi:10.1038/nm.2538
486 Willeit P, Zampetaki A, Dudek K, Kaudewitz D, King A, Kirkby NS, et al. Circulating microRNAs as novel
487 biomarkers for platelet activation. Circulation Res. 2013;112:595-600.
488 doi:10.1161/circresaha.111.300539
489 Zampetaki A, Willeit P, Tilling L, Drozdov I, Prokopi M, Renard JM, et al. Prospective study on circulating
490 MicroRNAs and risk of myocardial infarction. J Am Coll Cardiol. 2012:60:290-299.
491 doi:10.1016/j.jacc.2012.03.056
492 Zhang YY, Zhou X, Ji W J, Shi R, Lu R Y, Li JL, et al. Decreased circulating microRNA-223 level predicts high
493 on-treatment platelet reactivity in patients with troponin-negative non-ST elevation acute
494 coronary syndrome. J Thromb Thrombolysis. 2014;38:65-72. doi:10.1007/s11239-013-1022-9
495 Zhu H, Wu H, Liu X, Evans BR, Medina DJ, Liu CG, et al. Role of MicroRNA miR-27a and miR-451 in the
496 regulation of MDR1/P-glycoprotein expression in human cancer cells. Biochem Pharmacol. 2008;
497 76:582-588. doi:10.1016/j.bcp.2008.06.007
498 Zufferey A, Ibberson M, Reny JL, Nolli S, Schvartz D, Docquier M, et al. New molecular insights into
499 modulation of platelet reactivity in aspirin-treated patients using a network-based approach.
500 Hum Genet. 2016;135:403-414. doi:10.1007/s00439-016-1642-1

501

502

503

504

505

506

507

508

509

510

511

Figure Legend

Figure 1. miRNAs and aspirin in cardiovascular disease.

536

537 **Table 1. MiRNAs, cardiovascular disease and platelets**

Regulated miRNA	Cardiovascular disease or event	Target gene	References
miR-45	Ischemia stroke	CD40	(Guo et al., 2016)
miR-135a-5p and miR-204-5p	Platelet reactivity	THBS1, CDC42, CORO1C, SPTBN1, TPM3, GTPBP2, and MAPRE2	(Zufferey et al., 2016)
lower expression of miR-19b-1-5p	Aspirin insensitivity	-	(Zufferey et al., 2016)
miR-92a level and platelet distribution width (PDW) assay	Aspirin insensitivity	-	(H. G. Binderup et al., 2016)
miR-223	Platelet reactivity	-	(Chyrchel et al., 2015)
miR-126	Platelet levels altered by aspirin administration	CXCL12, PIK3R2, SPRED1	(Zufferey et al., 2016)

538

539

540

541

542

543

544

545

546