Table 1
The characteristics of the included studies.

Participants		APPLE, 2012 [5] (N = 221) Children and adolescents with SLE		LAPS, 2011 [6] (N = 200) Adult with SLE		Mok et al., 2011 [7] (N = 72) Adult with SLE	
Intervention	Medication	Atorvastatin 10–20 mg Qd	Placebo	Atorvastatin 40 mg Qd	Placebo	Rosuvastatin 10 mg Qd	Placebo
	Duration (months)	36	36	24	24	24	24
	Sample size (n)	113	108	99	101	36	36
Traditional cardiovascular risk factors	Age (years)	15.7 ± 2.8[a]	15.8 ± 2.5	44.7 ± 11.3		51.3 ± 10.4	50.3 ± 9.2
	Female (%)	84.1	82.4	90	94	94	100
	Body mass index (kg/m^2)	25.0 ± 5.2	23.8 ± 5.4	NA	NA	NA	
	Smoking (%)	2.7	3.7	NA	NA	NA	
	Hypertension (%)	34.2	34	49	48	39	28
	Diabetes (%)	NA	NA	4	5	6	0
	Cholesterol (mg/dl)	159.6 ± 41.1	150.6 ± 34.0	186 ± 38		185.6 ± 42.5	180.2 ± 37.5
	LDL-C (mg/dl)	91.8 ± 33.0	80.7 ± 28.7	103 ± 31		101.3 ± 40.2	93.6 ± 34.8
	HDL-C (mg/dl)	46.7 ± 12.9	46.0 ± 12.7	NA		57.2 ± 13.5	63.0 ± 16.2
	Triglyceride (mg/dl)	105.5 ± 52.8	122.9 ± 77.3	NA		141.0 ± 66.4	116.4 ± 61.8
SLE related factors	SLEDAI	4.92 ± 4.46	4.57 ± 4.07	2.2 (0–24)[b]	2.0 (0–18)	1.4 ± 1.5	1.8 ± 2.0
	Duration of SLE (months)	32.5 ± 29.1	29.4 ± 27.8	>10 years (48%)[c]	>10 years (34%)	12.4 ± 8.1	11.2 ± 5.9
Concomitant use of medications	Aspirin (%)	62.8	70.4	NA		50	50
	HCQ (%)	97.3	95.4	NA		39	53
	Corticosteroids (%)	81.3	82.4	NA		69	64
Baseline CIMT measurement (mm)	Max CIMT	0.579 ± 0.059	0.587 ± 0.053	NA		NA	
	Mean CIMT	0.465 ± 0.0439	0.471 ± 0.0409	0.59	0.57	0.68 ± 0.11	0.66 ± 0.15

LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; SLE: systemic lupus erythematosus; SLEDAI: systemic lupus erythematosus disease activity index; HCQ: hydroxychloroquine; CIMT: carotid intima-media thickness; NA: not available.
[a] Mean ± standard deviation.
[b] Median (range).
[c] Percentage of patients with duration > 10 years.

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Acetylsalicylic acid prevents platelet-induced proarrhythmic effects on intracellular Ca^{2+} homeostasis in ventricular myocytes

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Sudden cardiac death (SCD) is mostly caused by ventricular fibrillation (VF) during acute myocardial infarction (MI). MI results from thrombotic coronary occlusion. Previously, we reported that activated blood platelet products (ABPP) facilitate VF occurrence by increasing intracellular Ca^{2+} (Ca^{2+}_i) transients and L-type Ca^{2+} current ($I_{Ca,L}$) in cardiac myocytes [1]. Trypsin reduced these effects, suggesting involvement of peptides. Activated platelets release numerous substances including arachidonic-acid products and platelet-derived microvesicles (MV). Arachidonic-acid is released from phospholipids of the platelet plasma membrane. Cyclooxygenase converts arachidonic-acid into prostaglandins and thromboxane. These substances are involved in various processes, including platelet aggregation, vasoconstriction, and inflammation. Acetylsalicylic acid (ASA) blocks thromboxane-dependent platelet activation by inhibiting cyclooxygenase. The beneficial cardiovascular effects of ASA are mainly ascribed to its antithrombotic actions. Whether ASA has antiarrhythmic properties in humans is unknown. Globular membrane fragments called MV include plasma membrane-derived microparticles and body-derived exosomes, and are a major source of ligands released by activated platelets, containing hundreds of proteins, bioactive lipids, growth factors, and other compounds [2,3]. MV play an important pleiotropic role in many biological processes, e.g., hemostasis, maintenance of vascular health, and immunity [3]. However, their direct effect on ventricular myocytes is unknown. In our search for the arrhythmia-inducing agent(s) in ABPP, we focused here on cyclooxygenase products and MV.

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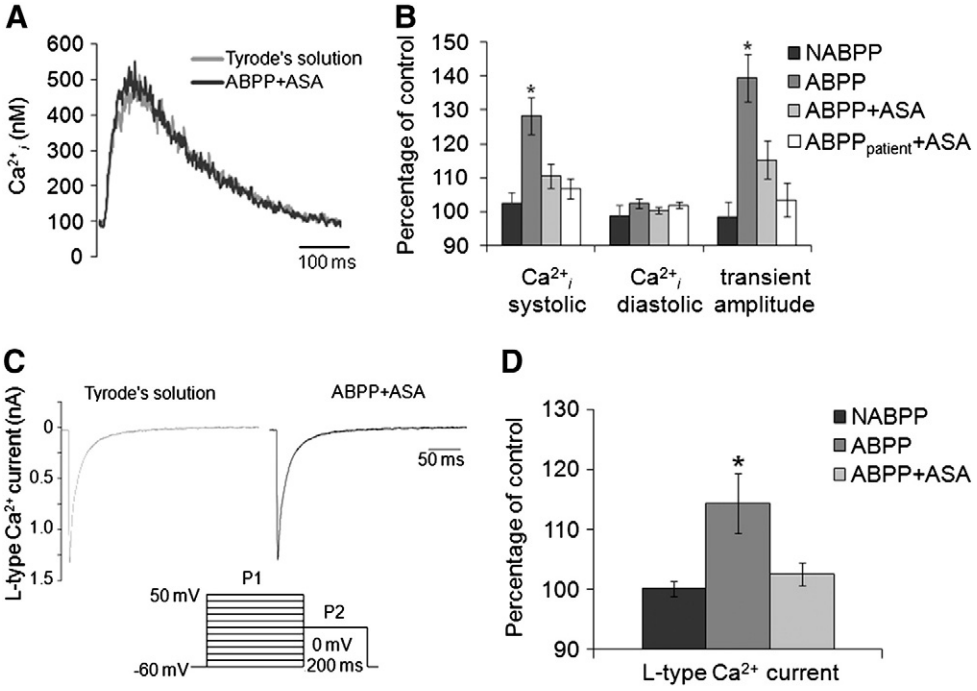

Fig. 1. ASA abolished the effects of ABPP. (A) Typical $Ca^{2+}{}_i$ transients in control condition and in the presence of ABPP + ASA. (B) Average $Ca^{2+}{}_i$ transient parameters. $^*P<0.05$ ABPP (n = 10) versus NABPP (n = 9), ABPP + ASA (n = 17) and $ABPP_{patient}$ + ASA (n = 10). (C) Representative $I_{Ca,L}$ activated upon depolarizing voltage steps to 0 mV in control conditions and in the presence of ABPP + ASA. Inset: protocol used. (D) Average $I_{Ca,L}$ measured at 0 mV. $^*P<0.05$ ABPP (n = 6) versus NABPP (n = 4) and ABPP + ASA (n = 14). Data are normalized to their control conditions, i.e., in the absence of NABPP, ABPP, ABPP + ASA or $ABPP_{patient}$ + ASA.

The study was approved by the institutional Medical Ethics Committee and the institutional animal experiments committee, and conforms to the principles outlined in the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals (NIH Publication 85–23, revised 1996). Informed consent was obtained from each subject. Blood platelets were isolated from fresh citrated platelet-rich-plasma of healthy volunteers (n = 3) and suspended to a concentration of 220–250×10^9/L in Tyrode's solution [1]. One portion of this suspension was filtrated with 0.45 μm pore-size syringe filters and snap-frozen in liquid nitrogen; the resulting solution contained non-activated blood platelet products (NABPP). The remaining portion of the suspension was activated by thrombin receptor activating protein and filtrated to obtain ABPP solution. Platelet activation was controlled by CD62p (α-granule membrane glycoprotein) and CD63 (lysosomal integral

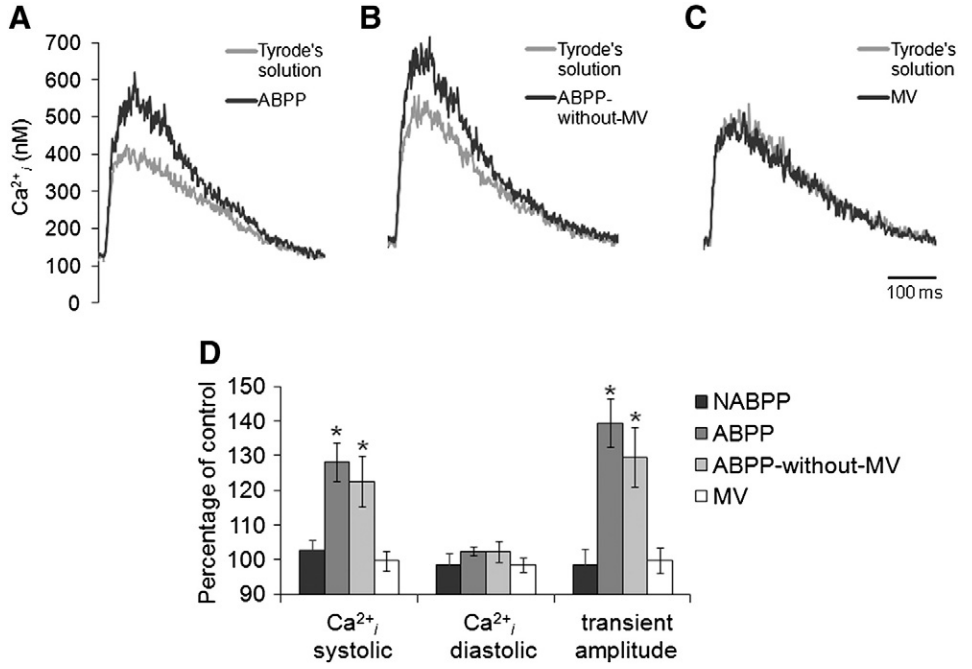

Fig. 2. MV had no influence on $Ca^{2+}{}_i$ transients. (A, B, C) Typical $Ca^{2+}{}_i$ transients in control conditions and in the presence of ABPP, ABPP-without-MV, and MV, respectively. (D) Average effects of NABPP (n = 9), ABPP (n = 10), ABPP-without-MV (n = 6), and MV (n = 5) on $Ca^{2+}{}_i$ parameters. $^*P<0.05$ ABPP versus NABPP and MV. Data are normalized to their control conditions, i.e., in the absence of NABPP, ABPP, ABPP-without-MV, or MV.

membrane protein) labeling. We inhibited cyclooxygenase by incubating platelet-rich-plasma with 0.02 mg/ml ASA for 15 min; this yielded ABPP + ASA or NABPP + ASA. Incubation with ASA had no effect on Ca^{2+}_i (data not shown). We also studied ASA effects in patients who experienced VF during MI (n = 2). In these patients, cyclooxygenase was inhibited by chronic ASA treatment following their MI. Accordingly, their platelet-rich-plasma was not incubated with ASA ($ABPP_{patient}$ + ASA). MV were isolated by centrifugating ABPP aliquots (1 ml) for 1 h at 154000 g and 4 °C [4]. ABPP without MV (ABPP-without-MV), present in the upper 0.5 ml, was harvested by aspiration. The remaining pellet, containing MV, was resuspended in 1 ml Tyrode's solution. Midmyocardial left ventricular myocytes of New Zealand white rabbits were enzymatically isolated [5]. Ca^{2+}_i was measured at 37 °C in indo-1 loaded myocytes stimulated at 2 Hz using field stimulation [6]. Myocytes were superfused with Tyrode's solution containing (mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 5.5, HEPES 5.0; pH 7.4 (NaOH). We analyzed diastolic and systolic Ca^{2+}_i concentrations, and Ca^{2+}_i transient amplitudes (Tr amplitude). $I_{Ca,L}$ was measured at 37 °C using the ruptured whole-cell configuration of the patch-clamp technique [1]. Extracellular solution for $I_{Ca,L}$ measurements contained (mM): TEA-Cl 145, CsCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 5.5, HEPES 5.0; pH 7.4 (NMDG-OH). Pipette solution for $I_{Ca,L}$ measurements contained (mM): CsCl 145, K_2-ATP 5.0, EGTA 10, HEPES 10; pH 7.2 (NMDG-OH). $I_{Ca,L}$ was measured in the presence of 0.25 mM 4,4′ diisothiocyanatostilbene-2,2′-disulfonic acid (to block Ca^{2+}-activated Cl^- current [7]) with a double-pulse protocol (Fig. 1C). During the first depolarizing pulse (P1), $I_{Ca,L}$ activates. This current was used to analyze $I_{Ca,L}$ amplitude and voltage-dependence of activation; the second pulse (P2) was used to measure voltage-dependence of inactivation. Voltage-dependence of (in)activation was determined by fitting a Boltzmann function $(y = [1 + \exp\{(V - V_{1/2})/k\}])^{-1}$ to the individual curves, yielding half-maximal voltage $V_{1/2}$ and slope factor *k*. Ca^{2+}_i and $I_{Ca,L}$ were measured in paired experiments, i.e., in the absence or presence of NABPP or ABPP (40× dilution) in the same myocyte. Data were normalized to the control condition, i.e., in the absence of NABPP or ABPP, and presented as mean ± SEM. Group comparisons were made using one-way repeated measures ANOVA followed by pairwise comparison using the Student–Newman–Keuls test. $P<0.05$ defined statistical significance.

In accordance with our previous study [1], we found that ABPP increased systolic Ca^{2+}_i, Tr amplitude, and $I_{Ca,L}$ amplitude significantly by 28%, 39%, and 14%, respectively, while NABPP did not affect these variables (Fig. 1). ASA strongly attenuated these effects (10%, 15%, and 3% increases, respectively). The effects of ASA were similar in VF patients ($ABPP_{patient}$ + ASA). Voltage-dependence of (in)activation of $I_{Ca,L}$ was not significantly affected by NABPP, ABPP or ABPP + ASA. The effects of ABPP-without-MV on systolic Ca^{2+}_i and Tr amplitude (22% and 29% increases, respectively) were similar to those of ABPP, while MV alone had no effects (Fig. 2). Because these findings suggest that MV had no effects on Ca^{2+}_i homeostasis, we did not test possible effects of MV on $I_{Ca,L}$, as Ca^{2+}_i homeostasis and $I_{Ca,L}$ are closely linked.

In summary, ASA pretreatment significantly reduced the effects of ABPP on Ca^{2+}_i and $I_{Ca,L}$, pointing to an important role of the cyclooxygenase pathway. In contrast, ABPP from which MV were removed had similar effects on Ca^{2+}_i as ABPP that contained MV, while MV alone had no effect on Ca^{2+}_i, indicating that MV are not involved. This narrows down the search for the agent(s) responsible for the proarrhythmic effects of ABPP, and eliminates hundreds of proteins present in MV [2,3]. Cyclooxygenase products include thromboxane and prostaglandins. Thromboxane A_2 is chemically unstable (it is hydrolyzed within ~30 s to biologically inactive thromboxane B2); thus, prostaglandins are more likely candidates responsible for the observed effects of ABPP. Our findings provide a mechanistic link between previous studies which showed that some cyclooxygenase products of arachidonic acid ($PGF_{2\alpha}$ and stable synthetic analog of thromboxane A_2) induce tachyarrhythmias in cultured neonatal rat myocytes [8], while ASA treatment afforded antiarrhythmic effects during balloon coronary occlusion in dogs [9]. These effects may be based on the molecular mechanism shown in the present study which is mediated by Ca^{2+}_i and $I_{Ca,L}$. Future studies must resolve whether ABPP of VF patients exert stronger effects on Ca^{2+}_i and $I_{Ca,L}$ than ABPP of patients who did not suffer VF during MI. To detect intrinsic differences between both patient categories, these studies must be conducted in the absence of ASA, because we demonstrated that ASA strongly attenuated the effects of ABPP on Ca^{2+}_i and $I_{Ca,L}$ (and that the effects of ASA-treated ABPP were similar in healthy volunteers and VF patients). Thus, ASA treatment may mask possible differences between both patient categories.

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References

[1] de Jong JSSG, Verkerk AO, van Borren MMGJ, et al. Activated human platelet products induce proarrhythmic effects in ventricular myocytes. J Mol Cell Cardiol 2011;51:347–56.
[2] Garcia BA, Smalley DM, Cho HJ, Shabanowitz J, Ley K, Hunt DF. The platelet microparticle proteome. J Proteome Res 2005;4:1516–21.
[3] Aatonen M, Grönholm M, Siljander PRM. Platelet-derived microvesicles: multitalented participants in intercellular communication. Semin Thromb Hemost 2012;38:102–13.
[4] Berckmans RJ, Sturk A, van Tienen LM, Schaap MCL, Nieuwland R. Cell-derived vesicles exposing coagulant tissue factor in saliva. Blood 2011;117:3172–80.
[5] Verkerk AO, Tan HL, Ravesloot JR. Ca^{2+}-activated Cl^- current reduces transmural electrical heterogeneity within the rabbit left ventricle. Acta Physiol Scand 2004;180:239–47.
[6] Baartscheer A, Schumacher CA, Belterman CNW, Coronel R, Fiolet JWT. SR calcium handling and calcium after-transients in a rabbit model of heart failure. Cardiovasc Res 2003;58:99–108.
[7] Verkerk AO, van Ginneken ACG, Berecki G, et al. Incorporated sarcolemmal fish oil fatty acids shorten pig ventricular action potentials. Cardiovasc Res 2006;70:509–20.
[8] Li Y, Kang JX, Leaf A. Differential effects of various eicosanoids on the production or prevention of arrhythmias in cultured neonatal rat cardiac myocytes. Prostaglandins 1997;54:511–30.
[9] Moschos CB, Haider B, De La Cruz C, Jr Lyons MM, Regan TJ. Antiarrhythmic effects of aspirin during nonthrombotic coronary occlusion. Circulation 1978;57:681–4.

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