(ECs). Our objective was to determine whether physiological concentrations of ADMA had a positive effect on NO production via CaSR stimulation.

Methods Inducible CaSR overexpressing HEK293 cells (Hek-CaSR) were generated using the FLP-IN T-REX transfection method. Intracellular Ca²⁺ mobilisation was assessed using the Ca²⁺ sensitive dye CAL520, while cAMP accumulation was measured using an ELISA kit. CaSR was stimulated by Gd³⁺ in the presence of 10µM ADMA. ADMA-CaSR signalling in ECs was investigated using human lung microvascular ECs (HuLMVECs). NO production was assessed using the fluorescent sensor DAF-AM. The CaSR antagonist NPS-2143 was used to show specificity to CaSR signalling.

Results Gd³⁺ releases stored intracellular Ca²⁺ and suppresses cAMP production in a dose dependent manner in HEK-CaSR cells. Incubation with 10µM ADMA for 1 hour prior to Gd³⁺ stimulation was found to sensitise CaSR and shift the dose-response curve for Ca²⁺ mobilisation leftward (CTRL EC₅₀ 0.16mM, ADMA EC₅₀ 0.05mM *p*=0.01). ADMA pre-treatment suppressed forskolin induced cAMP production to 70% of the maximal response. Ca²⁺ stimulation of HuLMVECs releases intracellular Ca²⁺ in a dose dependent manner, in the presence of 10µM ADMA maximal fluorescence is increased with no change to the EC₅₀. Given cytosolic Ca²⁺ release increases NOS activity we determined whether low concentrations of ADMA (10µM) promoted NO production. At maximal CaSR stimulation ADMA increased NO production by 40% (*p*=0.04). Consistent with previous reports higher concentrations of ADMA (100µM) blocked NO production through inhibition of NOS. The action of

ADMA was blocked by the addition of the CaSR inhibitor NPS-2143.

Conclusion Physiological concentrations of ADMA increase CaSR-mediated intracellular Ca²⁺ release and increase CaSR-mediated suppression of cAMP synthesis. In ECs, physiological concentrations of ADMA increase Ca²⁺ stimulated NO synthesis. In contrast pathophysiological concentrations directly inhibit EC NO synthase. These data suggest that physiological concentrations of ADMA positively regulate vascular reactivity while high concentrations of ADMA seen in cardiovascular disease reduce vascular function through NO blockade.

Conflict of interest None

BS42

HOW WARFARIN AND ANTIPLATELETS AFFECT CLOT STRUCTURE IN ATRIAL FIBRILLATION

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Introduction Atrial fibrillation (AF) and coronary artery disease (CAD) are two common cardiovascular conditions associated with increased morbidity and mortality. Both conditions bring a risk of thrombosis and thus antithrombotic therapy is indicated. Despite recognised treatment, stroke and myocardial infarctions still occur. Accordingly, the identification of patients whose haemostasis remains unimpaired by treatment is valuable.

Abstract BS42 Table 1 Demographic, clinical and haemostatic characteristic of patients with atrial fibrillation and coronary artery disease (control group)

		AF Warfarin n=184	AF Antiplatelets n=46	CAD n=40	p Kruskal- Wallis
Demographic and clinical characteristics					
Age, years		76 [67-80]	75 [71-83]	74 [61-78]	0.12
Males, n (%)		82 (45%)	12 (26%)	20 (50%)	0.04
Systolic BP, mmHg		130 [117-145]	130 [120-142]	139 [126-150]	0.08
Diastolic BP, mmHg		76 [68-80]	75 [64-85]	74 [65-80]	0.81
Body mass index, kg/m ²		29 [26-33] ^b	25 [23-30]	28 [24-31]	0.002
Smoking, n (%)		7 (4%)	3 (7%)	6 (15%)	0.02
Creatinine, n (%)		90 [75-121] ^a	90 [76-114]	82 [71-95]	0.04
eGFR, n (%)		66 [48-80] ^a	76 [52-87]	80 [63-88]	0.02
Creatinine clearance, n (%)		69 [45-85]	60 [46-85]	76 [57-96]	0.10
Coronary artery disease, n (%)		75 (41%)	17 (40%)	40 (100%)	<0.001
Diabetes, n (%)		76 (41%)	7 (15%)	17 (43%)	0.003
Hypertension, n (%)		151 (83%)	40 (87%)	37 (93%)	0.26
Chronic kidney disease, n (%)		64 (35%)	15 (33%)	4 (10%)	0.008
Peripheral artery disease, n (%)		17 (9%)	1 (2%)	0	0.04
Parameters of clot turbidity and lysis (non-normalised values)					
Maximum OD	PPP	0.55 [0.46-0.66]	0.52 [0.45-0.72]	0.56 [0.45-0.63]	0.78
	APPT	0.50 [0.43-0.63]	0.47 [0.42-0.63]	0.51 [0.41-0.59]	0.83
	Thrombin	0.58 [0.51-0.69]	0.58 [0.50-0.76]	0.56 [0.48-0.67]	0.62
Time to 50% maximum OD	PPP	454 [400-526] ^{ab}	328 [296-372] ^a	270 [238-318]	<0.001
	APPT	352 [318-398] ^{ab}	322 [288-360] ^a	280 [232-304]	<0.001
	Thrombin	268 [222-336] ^{ab}	232 [200-272] ^a	180 [146-220]	<0.001
Time between 50% clotting and 50% lysis	PPP	546 [456-700]	514 [464-604]	498 [436-598]	0.16
	APPT	626 [520-806] ^{ab}	562 [496-648]	558 [466-598]	<0.001
	Thrombin	560 [488-690]	530 [472-600]	534 [444-610]	0.04
Time to 50% lysis from maximum OD	PPP	360 [284-506]	332 [276-428]	334 [274-430]	0.30
	APPT	496 [398-692] ^a	484 [392-544]	462 [372-510]	0.03
	Thrombin	358 [306-500]	340 [296-400]	352 [288-422]	0.19

Purpose To investigate differences in plasma clot properties in patients with AF and CAD and compare the effect of warfarin and antiplatelets on clot structure in AF population.

Methods We studied 270 patients and divided them into 3 groups: AF on warfarin (n=184), AF on antiplatelets (n=46) and CAD (n=40). Plasma samples were obtained from participants and centrifuged to prepare platelet poor plasma. Assays were performed in 96-well polystyrene microtiter plates. Reagents were diluted in standard buffer (10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid [HEPES], pH 7.4, 150 mM NaCl). Patient plasma samples (25 %) were incubated with tissue plasminogen activator (500 ng.mL⁻¹) for 10 minutes at 37°C before the addition of CaCl₂ (7.5 mM). Either PPP reagent (2.5 %), aPPT reagent (2.5 %), or thrombin (0.5 U.mL⁻¹) were then added to initiate coagulation. Polymerisation of fibrin in plasma was monitored (ΔOD340 nm) using a Synergy H1 hybrid multi-mode plate reader, readings were taken in 12 second intervals for up to 60 minutes.

Results Comparisons between the 3 groups was performed using Kruskal-Wallis test, with Dunn's post-hoc analysis and Holm-Sidak adjustment. There were no significant differences in clot structure between 3 subgroups. The maximum rate of clot formation was significantly delayed in the warfarin subgroup with all reagents used (p<0.001) (table 1). Plasma clot susceptibility to fibrinolysis increased with warfarin compared to antiplatelets but was significant only with APPT and thrombin reagents (p<0.001 and 0.04 respectively).

Conclusion Warfarin was effective in delaying clot formation compared to antiplatelets and also resulted in increased susceptibility of plasma clot to fibrinolysis.

Conflict of interest None

BS43

ABSTRACT WITHDRAWN

BS44

CYTOKINE INDUCED DOWNREGULATION OF PLASMA MEMBRANE CALCIUM ATPASE 4 GENE INCREASES SENSITIVITY TO APOPTOSIS IN PULMONARY ARTERY ENDOTHELIAL CELLS

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Introduction Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by progressive vasoconstriction, vascular remodelling, and occlusion of small pulmonary arteries. It leads to increased pulmonary resistance and finally right ventricular failure. The disorder has no cure and current therapies only target vasoconstriction but have little effect on vessel remodelling. Increased activity of pro-inflammatory cytokines is linked to PAH pathogenesis. In this study, we analysed the effect of TNF-alpha and IL-1Beta on the expression of Plasma Membrane Calcium ATPase 4 (PMCA4) in pulmonary artery endothelial cells (PAEC).

Methods PAEC were cultured for different times and with different doses of TNF-alpha or IL-1Beta. Expression of PMCA4 RNA and protein was determined by qPCR and western blot respectively. PMCA4 expression was silenced using siRNA specific for human PMCA4. Quantification of apoptotic cells was performed by flow cytometry and TUNNEL.

Results Treatment of PAEC with TNF-alpha or IL-1Beta induced a time- and dose-dependent decrease in the levels of RNA for PMCA4. Analysis of PMCA4 RNA levels in the lungs of mice with overexpression of ectopic TNF-alpha confirmed the in vivo relevance of these observations. RNA decay experiments performed by blocking cellular transcription with Actinomycin D indicate that the downregulation of PMCA4 RNA levels mediated by pro-inflammatory stimuli in PAEC is the result of a decrease in RNA stability. In agreement with the reduction observed in RNA levels, PMCA4 protein expression was strongly decreased by treating PAEC with TNF-alpha or IL-1Beta. Silencing PMCA4 gene expression sensitised PAEC to apoptosis, suggesting that PMCA4 protects PAEC to apoptosis induced by pro-inflammatory cytokines.

Conclusion The pro-inflammatory cytokines TNF-alpha and IL-1Beta significantly downregulate the expression of the PMCA4 gene in PAEC at the RNA and protein level. Decrease in PMCA4 expression sensitised PAEC to apoptosis. This indicates that the PMCA 4 gene might play an important role in the apoptotic loss of endothelial cells observed in the pulmonary arterioles of patients with PAH.

Conflict of interest None

BS45

ACTIVATING TRANSCRIPTION FACTOR ATF2 NEGATIVELY REGULATES THE EXPRESSION OF ENDOTHELIAL NOTCH LIGANDS

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Introduction ATF2 also known as cyclic AMP response element binding protein 2 (CREB2) is a member of the leucine zipper (bZIP) transcription factor family that binds to specific DNA sequences and regulates the transcriptional activation of target genes. ATF2 regulation and functions have been studied in a number of developmental and pathological conditions. Studies have shown that activation of ATF2 by VEGF mediates angiogenic processes such as endothelial cell migration and tubular morphogenesis but the molecular role of ATF2 in these processes is largely unknown. To shed some light on this matter we are identifying ATF2-target genes induced by stimulation of endothelial cells with pro-angiogenic stimuli.

Methods HUVEC were stimulated with various pro-angiogenic stimuli at different times, and the activation (phosphorylation) status of ATF2 was determined by Western Blot.

ATF2 functionality in endothelial cells was suppressed by infecting HUVEC with an adenovirus encoding a phosphorylation-mutant, dominant-negative version of ATF2 (Ad-ATF2AA) where phosphorylation residues Thr69 and Thr71 have been mutated to Ala. HUVEC infection with an adenovirus encoding GFP was used as a control. PCR-based screening of specific gene arrays was used to identify the effect of ATF2 loss-