
Article:
Parker, W.A.E. orcid.org/0000-0002-7822-8852, Sagar, R., Kurdee, Z. et al. (5 more authors) (2021) A randomised controlled trial to assess the antithrombotic effects of aspirin in type 1 diabetes : role of dosing and glycaemic control. Cardiovascular Diabetology, 20 (1). 238.

https://doi.org/10.1186/s12933-021-01427-y

Cardiovascular Diabetology

ORIGINAL INVESTIGATION

Open Access

A randomised controlled trial to assess the antithrombotic effects of aspirin in type 1 diabetes: role of dosing and glycaemic control

William A. E. Parker[1], Rebecca Sagar[2], Zeyad Kurdee[2,3], Fladia Hawkins[2], Khalid M. Naseem[2], Peter J. Grant[2], Robert F. Storey[1†] and Ramzi A. Ajjan[2*†]

Abstract

Background: The enhanced thrombotic milieu in diabetes contributes to increased risk of vascular events. Aspirin, a key antiplatelet agent, has inconsistent effects on outcomes in diabetes and the best dosing regimen remains unclear. This work investigated effects of aspirin dose and interaction with glycaemia on both the cellular and protein components of thrombosis.

Methods: A total of 48 participants with type 1 diabetes and 48 healthy controls were randomised to receive aspirin 75 or 300 mg once-daily (OD) in an open-label crossover study. Light transmittance aggregometry and fibrin clot studies were performed before and at the end of each treatment period.

Results: Aspirin demonstrated reduced inhibition of collagen-induced platelet aggregation (PA) in participants with diabetes compared with controls, although the higher dose showed better efficacy. Higher aspirin dose facilitated clot lysis in controls but not individuals with diabetes. Collagen-induced PA correlated with glycaemic control, those in the top HbA1c tertile having a lesser inhibitory effect of aspirin. Threshold analysis suggested HbA1c levels of > 65 mmol/mol and > 70 mmol/mol were associated with poor aspirin response to 75 and 300 mg daily doses, respectively. Higher HbA1c was also associated with longer fibrin clot lysis time.

Conclusions: Patients with diabetes respond differently to the antiplatelet and profibrinolytic effects of aspirin compared with controls. In particular, those with elevated HbA1c have reduced inhibition of PA with aspirin. Our findings indicate that reducing glucose levels improves the anti-thrombotic action of aspirin in diabetes, which may have future clinical implications.

Trial registration: EudraCT, 2008-007875-26, https://www.clinicaltrialsregister.eu/ctr-search/search?query=2008-007875-26.

Keywords: Aspirin, Diabetes mellitus, Platelet inhibition, Fibrin

Introduction

Patients with diabetes mellitus, including type 1 (T1D) or type 2 (T2D), are at increased risk of atherothrombotic events, including acute coronary syndromes, thrombotic stroke and critical limb ischaemia [1, 2]. Platelet activation and fibrin network formation are key to the formation of intravascular obstructive thrombus in these conditions [3]. Aspirin (acetylsalicylic acid) is an

*Correspondence: R.Ajjan@leeds.ac.uk
†Robert F. Storey and Ramzi A. Ajjan contributed equally to the work
[2] Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK
Full list of author information is available at the end of the article

irreversible inhibitor of cyclo-oxygenase (COX) enzymes. By inhibiting platelet COX-1, aspirin blocks the generation of the potent pro-aggregatory and vasoconstrictive factor thromboxane (TX) A_2, whilst relatively sparing inhibition of anti-aggregatory and vasorelaxant prostacyclin, which may be synthesised in the endothelium by COX-1 or COX-2 depending on the setting [4]. As well as an antiplatelet effect, aspirin can acetylate fibrinogen, which affects fibrin network structure and facilitates fibrinolysis, a potential additional mechanism for the beneficial effects for aspirin following a cardiovascular event [5, 6].

Whilst there is good evidence for the use of aspirin in the secondary prevention of atherothrombotic events, the results of primary prevention trials in diabetes have led to controversy [7]. In the ASCEND trial (A Study of Cardiovascular Events in Diabetes), the most recent and largest study to date comprising 15,480 individuals with diabetes (94% with T2D) without pre-existing cardiovascular disease, aspirin therapy at a dose of 100 mg/day was associated with no net clinical benefit to justify its routine use in primary prevention [8].

It has been suggested that, in patients with diabetes, aspirin may be less efficacious than in people without diabetes, perhaps related to glycation of platelet proteins and/or clotting factors, making them less accessible for acetylation by the drug [9]. Similarly, accelerated platelet turnover in patients with T2D, remaining understudied in T1D, may be a further factor that reduces the efficacy of maintenance aspirin dosing [10].

One hypothetical strategy to mitigate any reduced efficacy of aspirin in those with diabetes may be to increase the dose. The current recommended dose of aspirin for cardiovascular prophylaxis is 75–100 mg once daily (OD) [11]. This is largely based on an analysis by the Antithrombotic Trialists' Collaboration, including a population with only a 4% prevalence of diabetes (of any type), that suggested higher doses offered no additional benefit in reducing ischaemic events [12]. There are also concerns that higher doses of aspirin may counterproductively inhibit endothelial prostacyclin release and lead to greater risk of gastroduodenal ulceration [13].

We hypothesised that high levels of glycation, caused by high average glycaemia, impair protein acetylation in diabetes and attenuate the antithrombotic effects of aspirin. Therefore, the aim of our work was to compare the effects of two different clinically-available doses of aspirin in patients with diabetes versus healthy controls in a randomised-controlled crossover trial and study the antithrombotic response both at the cellular and protein arms of blood coagulation. Individuals with type 1 diabetes (T1D) with no advanced complications were recruited into this trial to enable the study of potential interactions between glycation and acetylation away from other confounding variables commonly found in those with type 2 diabetes.

Methods

Study population

A single-centre, hospital outpatient-based randomised-controlled crossover trial was performed in 48 participants with T1D and 48 healthy controls. In the T1D group, participants had to be aged 18–50 years with a diagnosis of T1D treated with insulin only, using reliable contraception and not receiving any other medication. The control group met the same criteria apart from the fact they did not have a diagnosis of diabetes and were recruited from local hospital and university staff. Those with current or prior antithrombotic treatment or an indication for it, significant co-morbidity, receiving any drug other than insulin, with a contraindication to aspirin or, in the control group, evidence of diabetes were excluded. Full exclusion criteria are provided in Additional file 1.

Study treatments

Participants were randomised in an unrestricted manner to one of two medication sequences in a 1:1 crossover design (Fig. 1). Randomisation was performed by a member of staff outside the research team who sealed and shuffled envelopes containing an equal number of allocations to each sequence. Members of the research team then opened the envelopes and assigned participants to the directed sequence, cross-checked by pharmacy colleagues at time of drug prescription. Each participant received, in a randomised order, 14 days of aspirin 75 mg OD and 14 days of 300 mg OD. The two periods were separated by a three-week washout period. Study medication was open-label and was obtained from local hospital supplies of generic dispersible aspirin. A treatment period length of 14 days was chosen to ensure steady state of aspirin's effect and that there had been total platelet turnover during treatment [14], eliminating any chance of a carryover effect, whilst minimising drug exposure and maximising feasibility of the study. There were no significant changes to the study design after commencement.

Platelet function testing

The principal antithrombotic effect of aspirin is to reduce platelet activation, thus impairing aggregation. To study this, light transmittance aggregometry (LTA) was performed on platelet-rich plasma, prepared by centrifugation of citrated blood at 200 g for 10 min at room temperature, using collagen (2 μg/mL)

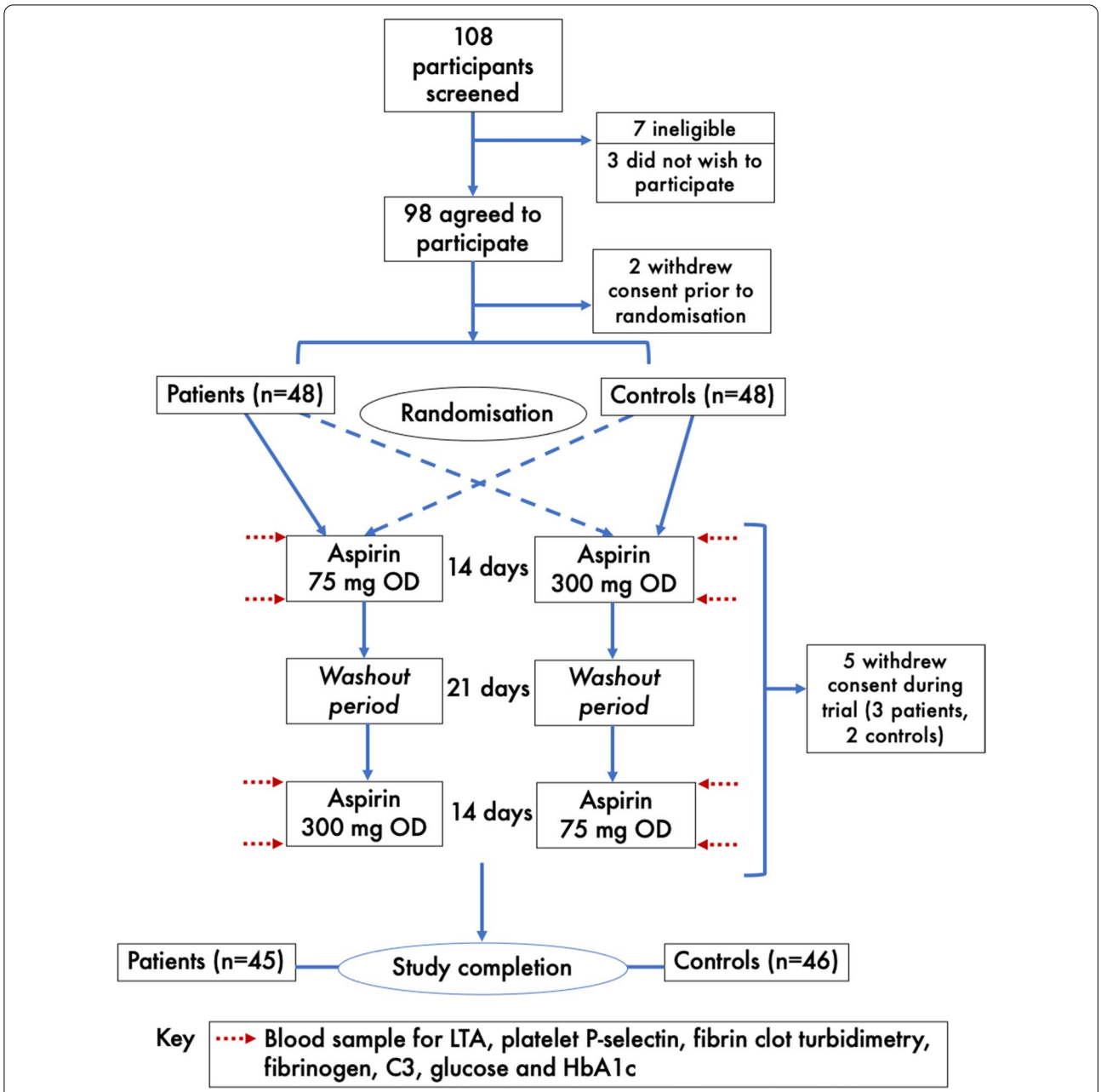

Fig. 1 Design of the study. C3, complement component 3; HbA1c, glycated haemoglobin A1c; LTA, light transmittance aggregometry; OD, once daily

and arachidonic acid (AA, 1 mmol/L) as agonists and a PAP-4 aggregometer (Bio/Data Corporation, Horsham, PA, USA), as previously described [15]. Maximum platelet aggregation (maxPA) responses at 6 min after addition of agonist, adjusted for baseline, were recorded. Samples were assessed in duplicate, taking the mean value for analysis, and repeated if a discrepancy of >10% was observed between the readings.

Fibrin clot turbidimetric analysis

As well as inhibiting platelets, aspirin may also have effects on the acellular arm of coagulation by modulating fibrin clot characteristics [5]. To assess the effect of aspirin dosing on fibrin clot dynamics, high-throughput turbidimetric analysis was performed as previously described and validated [16–20]. Briefly, lysis mix was added to citrated platelet-poor plasma samples before adding an activation mix that resulted in fibrin clot

formation and subsequent lysis. Serial absorbance was measured using an automated plate reader during clot formation until lysis was achieved. Variables recorded were lag time, representing the period from the addition of clot activation mix to the start of clot formation (a measure of clotting tendency), final clot turbidity (maximum absorbance, a representation of fibre thickness and clot density), and lysis time (time from full clot formation to 50% lysis, a measure of fibrinolysis potential).

Fibrinogen and complement C3

Fibrinogen levels, which can affect both clot formation and lysis, and can be associated with long-term cardiovascular risks in patients with diabetes [21], were determined by the clotting method of Clauss using a KC 10TM coagulometer (Henrich Amelung GmbH, Lemgo, Germany), as described elsewhere [22, 23]. Levels of complement C3, an inflammatory protein with anti-fibrinolytic properties, were determined, as previously described [18].

Statistical analysis

All participants who completed the study were included in the analysis set. The primary endpoint of the study was the change in maxPA response to collagen from start to end of each treatment period compared between patients with T1D and controls for each aspirin regimen using unpaired t-tests. The secondary endpoint was maxPA response to collagen stratified by HbA1c level. maxPA responses AA; fibrin clot dynamics; and fibrinogen and C3 levels were exploratory endpoints, as were other analyses performed to gain further understanding of the effects seen. A primary aim of the study was also to estimate the prevalence of poor aspirin response, using an established definition of on-treatment PA response > 80% compared with off-treatment level when assessed using 2 μg/mL-collagen as an agonist during LTA [24]. Correlations between continuous variables were assessed using the Pearson method. GraphPad PRISM v7, RStudio v1.1.456 and SPSS Statistics v25 were used for analyses. Corrections for multiple tests were not made and therefore the results of secondary and exploratory analyses are considered to be hypothesis-generating only.

Power calculation

Using previous data on platelet inhibition by aspirin treatment in patients with diabetes [20], a group size of 45 was deemed sufficient to detect a 10% difference in maxPA response to 2 μg/mL-collagen between groups with a power of 90% (at $p = 0.05$), based on the assumption that within-patient standard deviation of the response variable is 14%. The same number was enough to detect a 7% difference in clot final turbidity and 13% difference in lysis, given SD of the response variables at 10% and 19%, respectively (power of 90%, at $p < 0.05$) [25–27].

Trial conduct

The trial protocol was approved by the National Health Service Research Ethics Service and the Medicines and Healthcare Products Regulatory Agency. The study was sponsored and monitored by the University of Leeds. Written informed consent was obtained from each participant prior to any study activities. The trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki (1964).

Results

Of 108 potential participants screened, 101 were found to be eligible. Ninety-eight agreed to participate but 2 withdrew consent before starting the study and 5 during the trial, leaving a total of 45 patients with T1D and 46 controls completing the study and therefore being included in analysis of the results (Fig. 1). Baseline characteristics are shown and compared in Table 1. There were no significant within-subject differences in any study endpoint between baseline values at the start of each of the two treatment periods (Additional file 1: Tables S1, S2). Recruitment and follow-up took place over a period of 22 months, and the trial closed only once the recruitment target had been met and all follow-up completed. No Good Clinical Practice-defined serious adverse reactions occurred during the trial.

Platelet aggregation responses

There were no significant differences in baseline maxPA responses between controls and patients (Additional file 1: Table S1, Fig. 2).

In controls and patients, both doses of aspirin significantly reduced maxPA responses to collagen 2 μg/mL (Fig. 2). When receiving either dose, the reduction from baseline was less in patients than controls, with the difference being statistically significant when receiving aspirin 300 mg once daily but not 75 mg once daily. maxPA responses to 1 mmol/L-AA were significantly reduced in both groups by either aspirin dose, and there were no differences in the changes from baseline between patients and controls (Fig. 2). In patients, but not controls, maxPA responses to 1 mmol/L-AA, but not collagen 2 μg/mL, were significantly lower when receiving aspirin 300 mg once daily when compared to 75 mg OD (Additional file 1: Table S1, Fig. 2), though the mean difference was only – 0.51% (95% confidence interval – 0.98 to 0.04). When defined as on-treatment 2 μg/mL-collagen-induced PA response > 80% compared with off-treatment level, poor aspirin response was seen in 24% when

Table 1 Baseline characteristics of participants completing the study

	Controls (n = 46)	Patients (n = 45)	p value
Sex (female/male)	20/26 (43.5%/56.5%)	19/26 (42.2%/57.8%)	> 0.99
Age (years)	24.4 ± 6.4	26.0 ± 6.8	0.23
History of smoking, n (%)	2 (4.3%)	9 (20.0%)	0.03
Blood pressure			
Systolic (mmHg)	116.8 ± 12.3	114.8 ± 11.2	0.42
Diastolic (mmHg)	76.3 ± 9.7	78.7 ± 8.7	0.21
Physical examination			
Height (m)	1.73 ± 0.1	1.73 ± 0.1	0.74
Weight (kg)	69.9 ± 11.3	72.9 ± 9.9	0.17
BMI (kg/m^2)	23.2 ± 2.9	24.6 ± 3.5	0.04
Baseline blood tests			
HbA1c (mmol/mol)	35.2 ± 2.9	70.3 ± 17.7	< 0.0001
Sodium (mmol/L)	140.9 ± 1.9	139.8 ± 2.4	0.02
Potassium (mmol/L)	4.0 ± 0.3	4.1 ± 0.3	0.06
Creatinine (μmol/L)	80.9 ± 13.1	78.5 ± 20.7	0.50
Urea (mmol/L)	4.9 ± 1.2	5.3 ± 1.3	0.16
Bilirubin (μmol/L)	11.0 ± 6.5	11.9 ± 6.5	0.43
ALT (IU/L)	21.2 ± 8.4	19.3 ± 8.4	0.24
ALP (IU/L)	166.0 ± 48.5	203.5 ± 70.6	0.004
Albumin (g/L)	46.0 ± 2.4	44.7 ± 2.6	0.02
Total cholesterol (mmol/L)	4.2 ± 0.9	4.4 ± 0.7	0.23
LDL (mmol/L)	2.4 ± 0.8	2.5 ± 0.5	0.43
HDL (mmol/L)	1.5 ± 0.4	1.6 ± 0.4	0.24
Triglycerides (mmol/L)	0.96 ± 0.31	0.90 ± 0.35	0.34
FT_4 (pmol/L)	14.8 ± 2.2	15.0 ± 1.7	0.70
TSH (mIU/L)	2.4 ± 1.0	2.3 ± 1.3	0.84
Haemoglobin (g/dL)	14.2 ± 1.2	14.4 ± 1.0	0.22
Leukocyte count ($\times 10^9$/L)	6.1 ± 1.5	6.4 ±1.7	0.43
Platelet count ($\times 10^9$/L)	266 ± 53	279 ± 67	0.29
Time since diabetes diagnosis (months)	–	124.8 ± 101.8	–
Family history			
Ischaemic heart disease	16 (34.8%)	9 (20.0%)	0.16
Autoimmunity	11(23.9%)	16 (35.6%)	0.26

Where appropriate, controls and patients were compared using Fisher's exact test for proportions and two-tailed unpaired t-tests for continuous variables. Values for continuous variables are expressed as mean ± SD. ALP, alkaline phosphatase; ALT, alanine transferase; BMI, body mass index; FT_4, free thyroxine; Hb, haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TSH, thyroid stimulating hormone

receiving aspirin 75 mg OD and 22% when receiving 300 mg OD.

Fibrin clot characteristics

At baseline, there were no significant differences in fibrin clot characteristics when compared between controls and patients (Additional file 1: Table S2, Fig. 3). Similarly, there were no significant differences in the changes from baseline between the groups when receiving aspirin 75 mg OD (Fig. 3). However, when receiving aspirin 300 mg OD, change in final clot turbidity and lysis time was significantly more pronounced in controls than patients, the latter group displaying no effect. Neither aspirin nor diabetes status had any significant effect on clot lag time (Additional file 1: Table S2).

Plasma levels of fibrinogen and complement C3

Fibrinogen and C3 levels can affect fibrin clot characteristics [28], and therefore plasma levels of these proteins were investigated. No difference in fibrinogen or C3 levels were detected at baseline, comparing patients and controls (Additional file 1: Table S2). However, higher-dose aspirin resulted in reduced fibrinogen levels in controls, but not patients (Fig. 4). C3 levels were not affected by aspirin administration (Fig. 4).

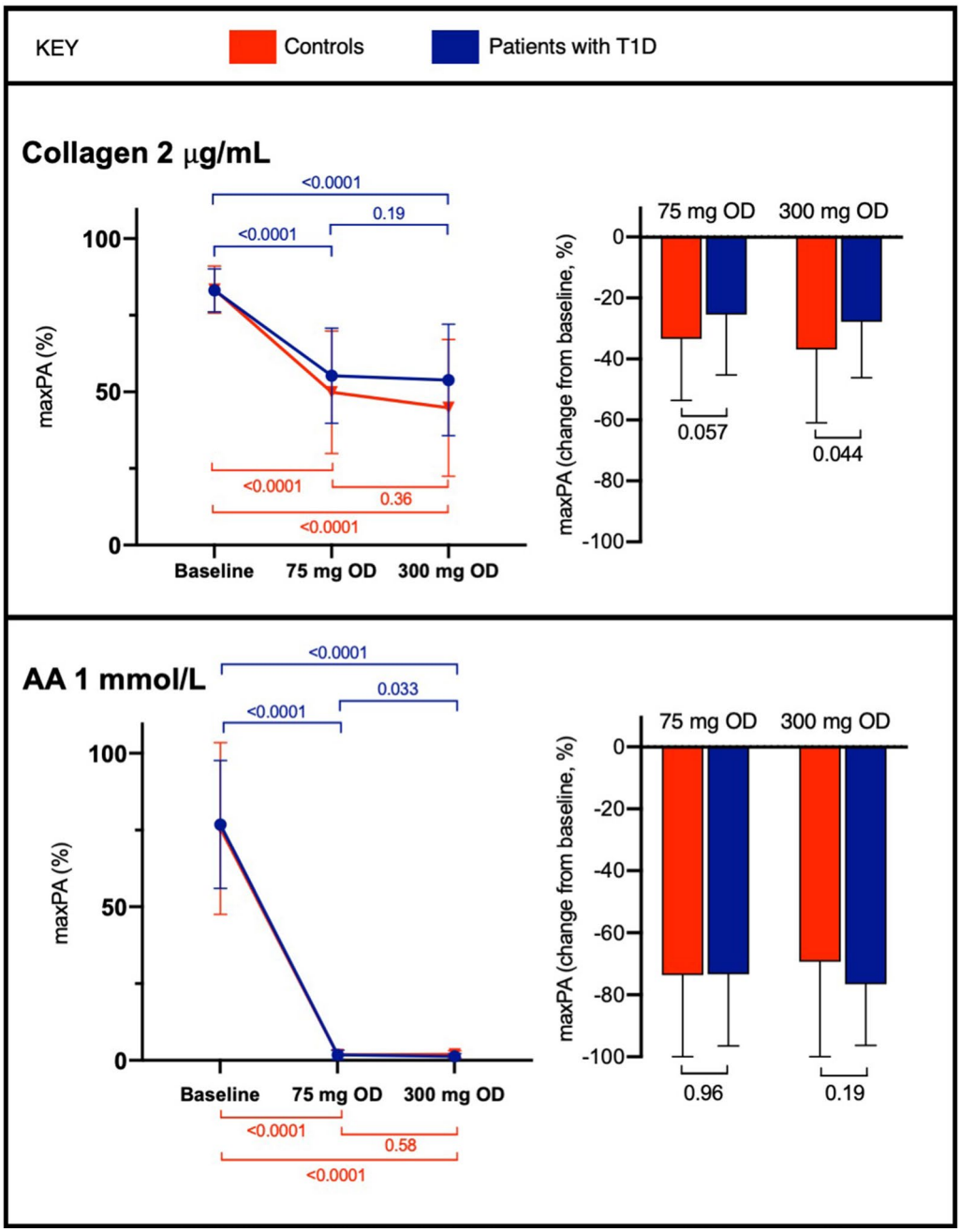

Fig. 2 Maximum platelet aggregation (maxPA) responses to collagen and arachidonic acid (AA) in controls and patients with type 1 diabetes (T1D) at baseline and when receiving aspirin 75 mg or 300 mg once daily (OD). Bars represent mean ± SD. P values are shown for within-participant comparisons (left) and between groups (right)

Correlation with glycation and glycaemic status in patients with T1DM

Levels of glycated haemoglobin A1c (HbA1c) had no significant correlation with maxPA responses at baseline or when receiving aspirin 75 mg OD (Table 2; Fig. 5). When receiving 300 mg OD, there was a significant, positive correlation between HbA1c and maxPA responses to collagen (Table 2; Fig. 5). Further analysis, however, suggested no significant correlation between HbAlc and difference in collagen-induced maxPA responses when

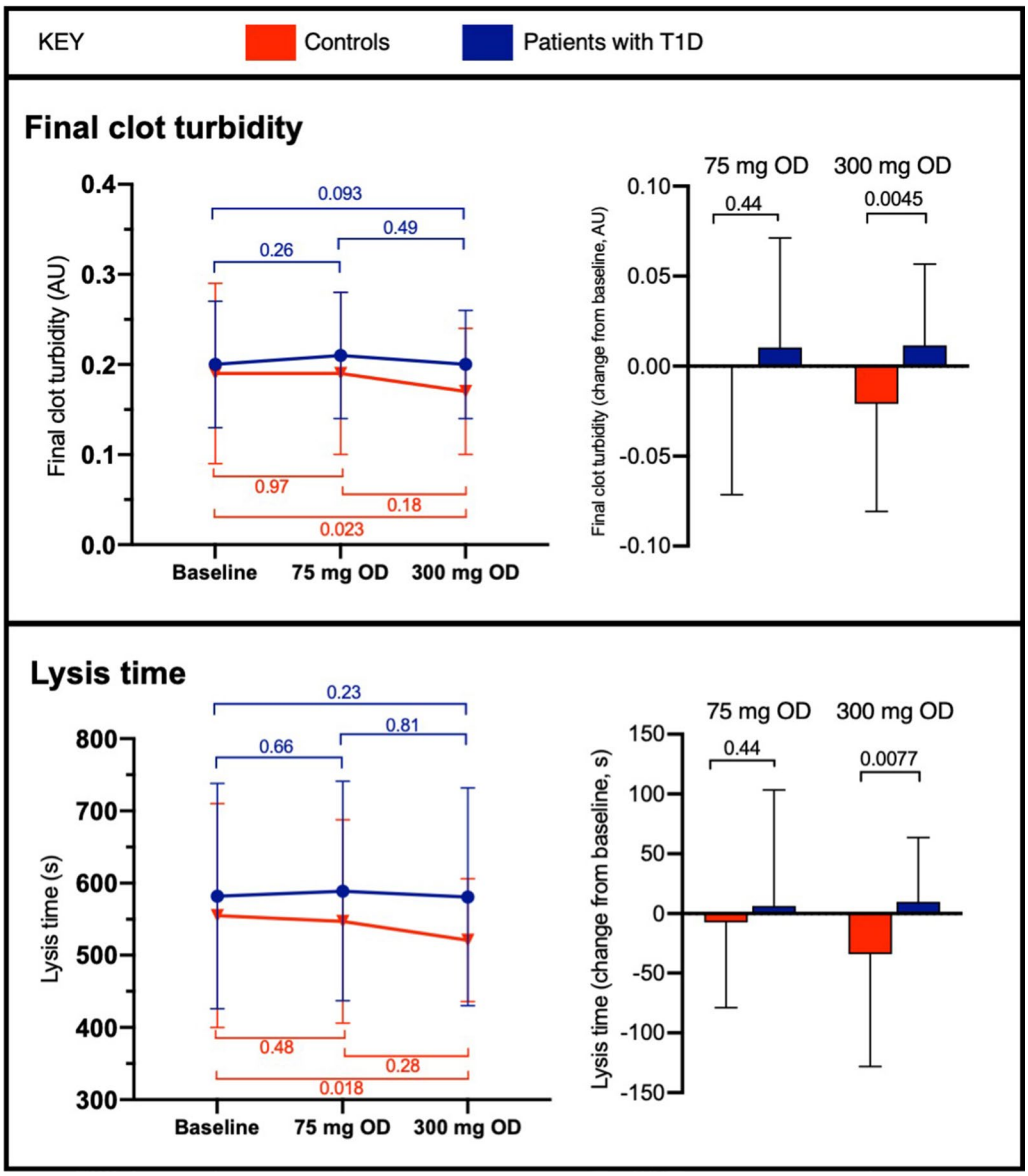

Fig. 3 Fibrin clot final turbidity and lysis time in controls and patients with type 1 diabetes (T1D) at baseline and when receiving aspirin 75 mg or 300 mg once daily (OD). Bars represent mean ± SD. P values are shown for within-participant comparisons (left) and between groups (right)

receiving aspirin 75 mg OD vs. 300 mg OD (Additional file 1: Fig. S1).

Regarding fibrin clot dynamics, at baseline and when receiving aspirin 75 or 300 mg OD, HbA1c positively and significantly correlated with final clot turbidity and lysis time (Table 2; Fig. 5). There was no significant correlation with lag time at any timepoint.

Levels of HbA1c positively and significantly correlated with plasma fibrinogen at baseline and when receiving either aspirin dose (Table 2). No correlation between C3 levels and HbA1c was observed at baseline or during treatment with aspirin.

At baseline, there was a positive and significant correlation between plasma glucose and fibrinogen levels as well as lag time (Table 2). During aspirin treatment at either dose, there was no significant correlation between plasma glucose and any study endpoint.

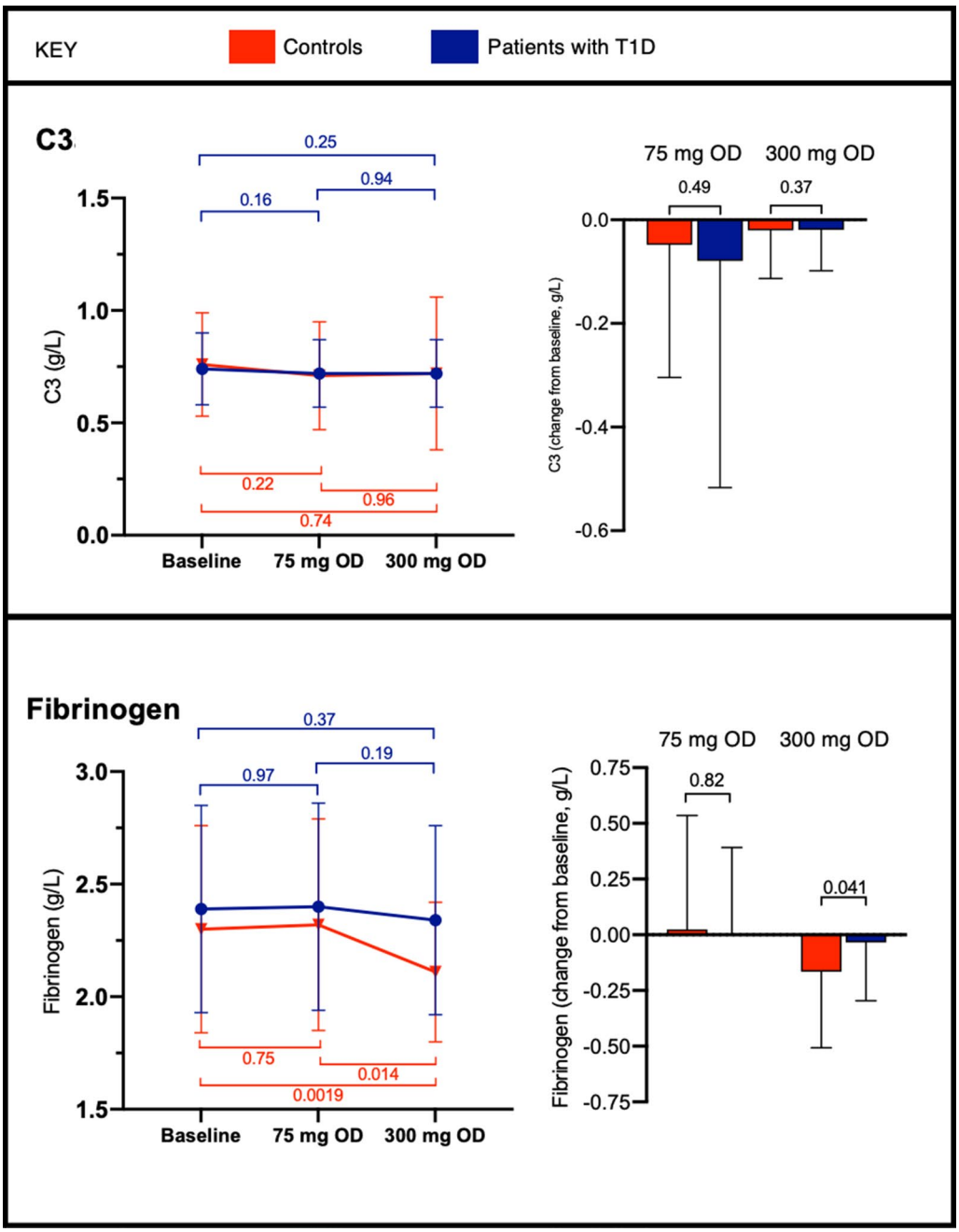

Fig. 4 Plasma fibrinogen and complement component 3 (C3) levels in controls and patients with type 1 diabetes (T1D) at baseline and when receiving aspirin 75 mg or 300 mg once daily (OD). Bars represent mean ± SD. P values are shown for within-participant comparisons (left) and between groups (right)

Threshold analysis of the relationship between HbA1c and poor aspirin response

To further explore the influence of glycation status on response to aspirin in patients with diabetes, tertiles of HbA1c were investigated in relation to collagen-induced PA responses. Whether receiving aspirin 75 mg OD or 300 mg OD, those in the highest tertile had less inhibitory effect of aspirin (Fig. 6).

The ability of HbA1c to predict poor response to aspirin treatment was also assessed using a binary logistic regression model. Poor response to aspirin has been defined as an on-treatment PA response > 80%

Table 2 Correlation between glycaemic control, measured as haemoglobin (Hb)A1c, plasma glucose levels and study endpoints in type 1 diabetes patients at baseline and when receiving aspirin 75 mg or 300 mg once daily (OD)

Parameter	Correlation with HbA1c						Correlation with plasma glucose					
	Baseline		75 mg OD		300 mg OD		Baseline		75 mg OD		300 mg OD	
	R	p	R	p	R	p	R	p	R	p	R	p
Platelet aggregation												
AA 1 mmol/L	0.24	0.11	0.17	0.28	0.13	0.41	0.058	0.71	0.12	0.47	0.054	0.73
Collagen 2 µg/mL	0.095	0.55	0.25	0.11	**0.36**	**0.019**	− 0.13	0.42	0.032	0.84	0.096	0.54
Collagen 16 µg/mL	0.076	0.62	− 0.015	0.93	0.23	0.14	0.15	0.33	− 0.06	0.71	0.068	0.67
Fibrin clot turbidimetry												
Lag time (s)	0.17	0.27	0.0032	0.98	− 0.11	0.48	0.36	0.019	0.044	0.79	0.046	0.77
Final clot turbidity (AU)	**0.57**	**< 0.0001**	**0.54**	**0.00021**	**0.45**	**0.0033**	0.2	0.19	0.31	0.05	0.24	0.12
Lysis time (s)	**0.54**	**0.00015**	**0.38**	**0.011**	**0.4**	**0.01**	0.046	0.77	0.059	0.71	0.21	0.17
Fibrinogen and C3												
Fibrinogen (g/L)	**0.48**	**0.00099**	**0.32**	**0.034**	**0.48**	**0.0017**	**0.35**	**0.022**	0.09	0.57	0.18	0.24
C3 (g/L)	0.24	0.12	0.21	0.18	0.094	0.56	0.011	0.94	0.097	0.55	− 0.24	0.13

Data generated using the Pearson method. Values in bold indicate those associated with a p value < 0.05

compared with off-treatment level when assessed using 2 µg/mL-collagen as an agonist during LTA [24]. Simple logistic regression was used to identify factors associated with poor aspirin response when receiving aspirin 75 mg OD or 300 mg OD (Additional file 1: Table S3). Testing threshold values of HbA1c ascending in 5 mmol/mol steps suggested that an HbA1c of >65 mmol/mol was significantly associated with poor aspirin response when receiving aspirin 75 mg OD (exp[B] = 5.70, p = 0.042) and >70 mmol/mol when receiving 300 mg OD (exp[B] = 6.77, p = 0.027). Receiver operating characteristic curves were constructed to further explore the ability of HbA1c level to predict poor aspirin response (Fig. 6). These demonstrated fair predictive value when receiving either 75 mg OD (area under curve [AUC] = 0.70) or 300 mg OD (AUC = 0.74). The cut-off of >65 mmol/mol when receiving 75 mg OD offered a sensitivity of 82% and specificity of 56%, whereas >70 mmol/mol when receiving 300 mg OD offered 80% sensitivity and 69% specificity.

Using a previously described approach [29], including variables with a univariate p value of <0.15 in a multivariate logistic regression model for each regimen, suggested that the threshold of >70 mmol/mol when receiving 300 mg OD was an independent predictor of poor aspirin response (exp[B] = 5.87, p = 0.045). However, the threshold of >65 mmol/L when receiving 75 mg OD did not reach a significant statistical level to demonstrate this (exp[B] = 3.77, p = 0.16, Additional file 1: Table S4–S5).

Discussion

It is generally believed that a daily aspirin dose of 75–100 mg is sufficient to maximally inhibit platelet COX-1 activity [13]. However, this has been based on findings from cohorts with a low incidence of diabetes, a condition that may hypothetically reduce the effectiveness of aspirin through glycation of its target proteins [9]. Our data suggest that aspirin was not as effective as reducing PA in patients with T1D compared to controls, particularly when receiving 300 mg OD. Whilst increasing aspirin dose from 75 mg OD to 300 mg OD offered significantly greater inhibition of AA-induced in patients with T1D, the difference was small and collagen-induced responses were similar. Combined with the fact that higher-dose aspirin may hypothetically increase counteractive effects on endothelial prostacyclin release, gastroduodenal integrity and haemostasis, which were not assessed in this study, it seems unlikely that this would translate into a clinical benefit in this patient group [30]. This is supported by recent data on clinical outcomes from Aspirin Dosing: A Patient-Centric Trial Assessing Benefits and Long-Term Effectiveness (ADAPTABLE), in which the aspirin regimens 81 mg OD and 325 mg OD were compared in 15,076 patients with established atherosclerotic cardiovascular disease [31]. After a median of 26 months, there was no significant difference in the rates of a composite primary endpoint of all-cause death, hospitalisation for myocardial infarction or hospitalisation for stroke (7.28% [81 mg] vs. 7.51% [325 mg]; hazard ratio 1.02; 95% confidence interval 0.91 to 1.14; p = 0.75). Furthermore, this finding appeared replicated

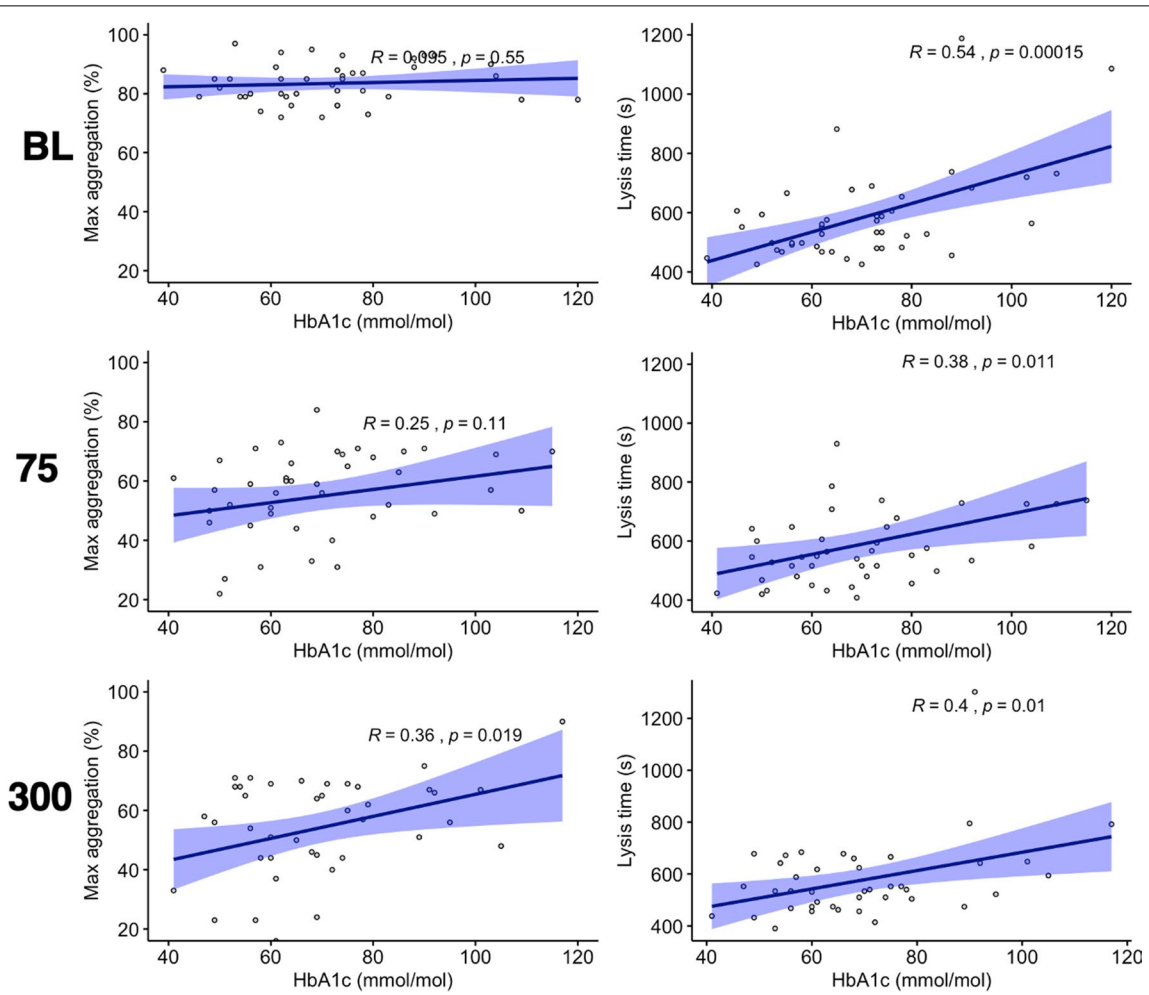

Fig. 5 Correlation between glycated haemoglobin (Hb)A1c and either maximum platelet aggregation response to collagen 2 µg/mL (left) or fibrin clot lysis time (right) at baseline (BL, top) and during treatment with aspirin 75 mg once daily (75, middle) or 300 mg once daily (300, bottom) in patients with type 1 diabetes. Solid blue line represents line of regression and shaded area represents 95% CI

in the subgroup (n = 5676) with diabetes (HR 0.99 [0.84 to 1.17]).

Fibrin clot dynamics may also be an important determinant of thrombotic risk. For example, lysis time is an independent predictor of cardiovascular risk after an acute coronary syndrome event, including in those with diabetes [6]. Fibrin clot lysis time was shortened and clot maximum absorbance reduced following 300 mg OD aspirin in controls, consistent with previous work using aspirin 150 mg OD [5], while 75 mg OD had no effect. In contrast, neither dose of aspirin affected lysis or clot maximum turbidity in those with T1D; therefore aspirin failed to modulate fibrin clot properties in our patient population (at least using 75 and 300 mg daily doses). Interestingly, fibrinogen levels were reduced by 300 mg aspirin in controls but not diabetes patients, providing one mechanism for the observed changes in fibrin clot parameters. This may be due to an anti-inflammatory effect for the higher aspirin dose, which appears to only affect individuals without diabetes. Work on the fibrin network in T1D is limited with a previous study showing that only higher dose aspirin (320 mg OD) affects fibrin clot permeability in these individuals, which was more pronounced in those with poor glycaemic control, but fibrin clot lysis was not studied [32]. However, we did not investigate clot permeability given that the role of this fibrin parameter in predisposition to cardiovascular events is unknown.

There was evidence of correlation between platelet function or fibrin clot dynamics during aspirin treatment and HbA1c but not plasma glucose. Furthermore, we determined that poor response to aspirin was particularly associated with an HbA1c level >65–70

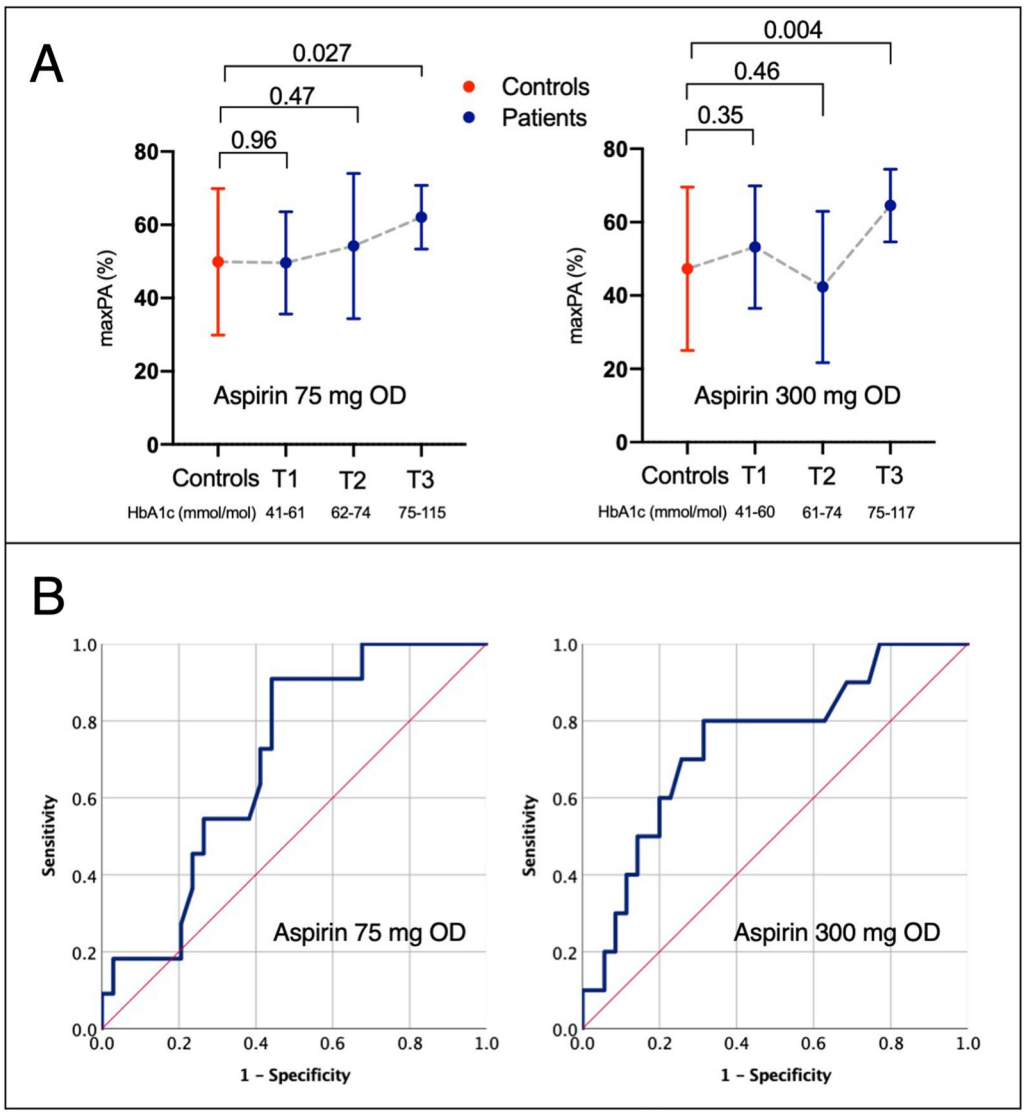

Fig. 6 Relationship between haemoglobin (Hb)A1c and collagen-induced maximum platelet aggregation (maxPA) responses when receiving aspirin 75 mg or 300 mg once daily (OD), presented by HbA1c tertile (T) (panel **A**, p values shown for controls vs. tertile) and as receiver operating characteristic curves for predicting poor response to aspirin (panel **B**)

mmol/mol, those with a lower level having similar responses to controls. This supports the hypothesis that aspirin may be blocked from acetylating target proteins if these are glycated, rather than merely in the presence of hyperglycaemia. Our findings demonstrate that increasing aspirin dose from 75 mg OD to 300 mg OD does not overcome poor response in patients with high glucose levels, and, above all, our findings support the importance of good glycaemic control in reducing thrombotic risk. Our data suggest that avoiding an overly elevated level HbA1c may improve the antiplatelet response to aspirin and confer more advantageous fibrin clot dynamics. Further work is required to explore this hypothesis.

Beyond a dose increase, other hypothetical strategies for improving the strength and reliability of aspirin's antithrombotic effect in patients with diabetes might include adding a second antiplatelet agent such as a $P2Y_{12}$ inhibitor (dual antiplatelet therapy, DAPT). Though there is evidence that diabetes patients with established coronary artery disease may gain benefits of reduced ischaemic risk from long-term DAPT compared to aspirin alone [33, 34], there is currently no evidence for use of $P2Y_{12}$ inhibitors for primary prevention

of cardiovascular events in any population, including in those with diabetes. An alternative similarly unexplored regimen for primary prevention in patients with diabetes might be to combine aspirin with a low-dose anticoagulant drug such as the non-vitamin K antagonist oral anticoagulant, rivaroxaban [35]. As well as aspirin dose, increased frequency of dosing beyond OD may hypothetically improve aspirin exposure and counter high platelet turnover in this group, though whether this would overcome poor aspirin response in patients with very high HbA1c has not been well studied [36]. Intensifying antithrombotic therapy typically attracts a penalty of increased bleeding risk that would need to be carefully balanced to prove net clinical benefit in any trial in this population. Other approaches such as $P2Y_{12}$ inhibitor monotherapy or very-low-dose twice-daily aspirin-based regimens may plausibly help to better achieve an optimum balance of benefits and risks [37, 38]. Further study of alternative treatment options to standard doses of OD aspirin for primary prevention in patients with diabetes is needed.

There are clear strengths to this study that should be highlighted. First, we recruited young T1D individuals, thus limiting confounders, which allowed the assessment of the role of glycaemia in response to aspirin treatment. Second, the work studied both the cellular and protein arms of coagulation, thereby giving a comprehensive assessment of thrombosis potential. Finally, withdrawal rate was low with over 90% randomised participants completing the study. A limitation of this study is that levels of TXA_2 and prostacyclin release were not directly assessed. The study did not assess clinical outcomes of ischaemic or bleeding events, but is valuable in providing mechanistic insights into the findings of trials that have done so. We cannot be sure that our findings are applicable to patients with T2D, but given hyperglycaemia and hyperglycation are common to both T1D and T2D, it appears rational to expect this to be the case, though study specifically in T2D would be needed to definitively confirm it.

Conclusions

Patients with diabetes have an impaired response to aspirin compared to healthy controls with reduced platelet inhibition and absent profibrinolytic activity. Increasing the dose of aspirin in individuals with diabetes had little effect on the cellular and protein arms of coagulation, and the reduced response to this agent appears to be related to inadequate diabetes control. Therefore, improving glycaemia may be necessary to optimise the anti-thrombotic effect of aspirin in diabetes.

Abbreviations

AA: Arachidonicacid; ALP: Alkalinephosphatase; ALT: Alaninetransferase; ASCEND: AStudy of Cardiovascular Events in Diabetes; AUC: Areaunder curve; C3: Complementcomponent 3; COX: Cyclo-oxygenase; FT4: Freethyroxine; HbA1c: HaemoglobinA1c; HDL: High-densitylipoprotein; LDL: Low-densitylipoprotein; LTA: Lighttransmittance aggregometry.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-021-01427-y.

Additional file 1. Additional details of study design and further data analyses.

Acknowledgements

W.A.E. Parker is funded by a British Heart Foundation Clinical Training Research Fellowship (FS/18/49/33752).

Authors' contributions

The study was conceived by PJG, RFS and RAA, and supervised by RFS and RAA. FAH undertook the laboratory analysis, helped by ZK. WAEP performed statistical analysis, collated results and produced the first draft of the manuscript. All authors read and approved the final manuscript.

Funding

This study was funded by the British Heart Foundation (PG/09/020/26305). The funder had no input into running of the study, data collection or interpretation of the results.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its Additional file 1.

Declarations

Ethics approval and consent to participate

This trial was approved by the National Health Service Research Ethics Service prior to commencement.

Consent for publication

Not applicable.

Competing interests

RF Storey reports institutional research grants/support from AstraZeneca, Cytosorbents and GlyCardial Diagnostics; consultancy fees from AstraZeneca, Bayer, Bristol Myers Squibb/Pfizer, CSL Behring, Cytosorbents, GlyCardial Diagnostics, Hengrui, Idorsia, Novartis, PhaseBio, Portola, Sanofi Aventis and Thromboserin; and honoraria from AstraZeneca, Bayer, Bristol Myers Squibb/Pfizer, Intas Pharmaceuticals and Medscape. RA. Ajjan. received honoraria and educational and research support from Abbott Diabetes Care, AstraZeneca, Novo Nordisk, Eli Lilly, Bayer, Sanofi, MSD, and Boehringer Ingelheim. The other authors report no relevant disclosures.

Author details

[1]Cardiovascular Research Unit, Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, UK. [2]Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK. [3]Clinical Biochemistry Unit, Pathology Department, College of Medicine, King Saud University, Riyadh, Saudi Arabia.

Received: 14 October 2021 Accepted: 4 December 2021
Published online: 17 December 2021

References

1. Olesen KKW, Madsen M, Gyldenkerne C, Thrane PG, Thim T, Jensen LO, Bøtker HE, Sørensen HT, Maeng M. Ten-year cardiovascular risk in diabetes patients without obstructive coronary artery disease: a retrospective Western Denmark cohort study. Cardiovasc Diabetol. 2021; 20(1):23.
2. de Ferranti SD, de Boer IH, Fonseca V, Fox CS, Golden SH, Lavie CJ, Magge SN, Marx N, McGuire DK, Orchard TJ, et al. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association. Diabetes Care. 2014;37(10):2843.
3. Parker WAE, Storey RF. Antithrombotic therapy for patients with chronic coronary syndromes. Heart 2021; 107:925–933.
4. Kirkby NS, Lundberg MH, Harrington LS, Leadbeater PD, Milne GL, Potter CM, Al-Yamani M, Adeyemi O, Warner TD, Mitchell JA. Cyclooxygenase-1, not cyclooxygenase-2, is responsible for physiological production of prostacyclin in the cardiovascular system. Proc Natl Acad Sci U S A. 2012; 109(43):17597–17602.
5. Ajjan RA, Standeven KF, Khanbhai M, Phoenix F, Gersh KC, Weisel JW, Kearney MT, Ariens RA, Grant PJ. Effects of aspirin on clot structure and fibrinolysis using a novel in vitro cellular system. Arterioscler Thromb Vasc Biol. 2009; 29(5):712–717.
6. Sumaya W, Wallentin L, James SK, Siegbahn A, Gabrysch K, Himmelmann A, Ajjan RA, Storey RF. Impaired fibrinolysis predicts adverse outcome in acute coronary syndrome patients with diabetes: a PLATO sub-study. Thromb Haemost. 2020;120(3):412–22.
7. Seidu S, Kunutsor SK, Sesso HD, Gaziano JM, Buring JE, Roncaglioni MC, Khunti K. Aspirin has potential benefits for primary prevention of cardiovascular outcomes in diabetes: updated literature-based and individual participant data meta-analyses of randomized controlled trials. Cardiovasc Diabetol. 2019; 18(1):70.
8. Bowman L, Mafham M, Wallendszus K, Stevens W, Buck G, Barton J, Murphy K, Aung T, Haynes R, Cox J, et al. Effects of aspirin for primary prevention in persons with diabetes mellitus. N Engl J Med. 2018;379(16):1529–39.
9. Ajjan R, Storey RF, Grant PJ. Aspirin resistance and diabetes mellitus. Diabetologia. 2008; 51(3):385–390.
10. Vernstrom L, Funck KL, Grove EL, Laugesen E, Baier JM, Hvas AM, Poulsen PL. Antiplatelet effect of aspirin during 24 h in patients with type 2 diabetes without cardiovascular disease. Thromb Res. 2018; 161:1–6.
11. Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, Prescott E, Storey RF, Deaton C, Cuisset T, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J. 2020;41(3):407–77.
12. Antiplatelet Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ. 2002; 324(7329):71–86.
13. Patrono C, Morais J, Baigent C, Collet JP, Fitzgerald D, Halvorsen S, Rocca B, Siegbahn A, Storey RF, Vilahur G. Antiplatelet agents for the treatment and prevention of coronary atherothrombosis. J Am Coll Cardiol. 2017;70(14):1760–76.
14. Leeksma CHW, Cohen JA. Determination of the life span of human blood platelets using labelled diisopropylfluorophosphanate. J Clin Invest. 1956; 35(9):964–969.
15. Storey RF, Husted S, Harrington RA, Heptinstall S, Wilcox RG, Peters G, Wickens M, Emanuelsson H, Gurbel P, Grande P, et al. Inhibition of platelet aggregation by AZD6140, a reversible oral P2Y12 receptor antagonist, compared with clopidogrel in patients with acute coronary syndromes. J Am Coll Cardiol. 2007;50(19):1852–6.
16. Neergaard-Petersen S, Ajjan R, Hvas AM, Hess K, Larsen SB, Kristensen SD, Grove EL. Fibrin clot structure and platelet aggregation in patients with aspirin treatment failure. Plos One. 2013; 8:e71150.
17. Carter A, Cymbalista C, Spector T, Grant P. Heritability of clot formation, morphology, and lysis: the EuroCLOT study. Arterioscler Thromb Vasc Biol. 2007; 27:2783–2789.
18. Franchi F, Rollini F, Cho JR, King R, Phoenix F, Bhatti M, DeGroat C, Tello-Montoliu A, Zenni MM, Guzman LA, et al. Effects of dabigatran on the cellular and protein phase of coagulation in patients with coronary artery disease on dual antiplatelet therapy with aspirin and clopidogrel. Results from a prospective, randomised, double-blind, placebo-controlled study. Thromb Haemost. 2016;115(3):622–31.
19. Hess K, Alzahrani SH, Price JF, Strachan MW, Oxley N, King R, Gamlen T, Schroeder V, Baxter PD, Ajjan RA. Hypofibrinolysis in type 2 diabetes: the role of the inflammatory pathway and complement C3. Diabetologia. 2014; 57(8):1737–1741.
20. Parker WAE, Schulte C, Barwari T, Phoenix F, Pearson SM, Mayr M, Grant PJ, Storey RF, Ajjan RA. Aspirin, clopidogrel and prasugrel monotherapy in patients with type 2 diabetes mellitus: a double-blind randomised controlled trial of the effects on thrombotic markers and microRNA levels. Cardiovasc Diabetol. 2020; 19(1):3.
21. Yuan D, Jiang P, Zhu P, Jia S, Zhang C, Liu Y, Liu R, Xu J, Tang X, Zhao X, et al. Prognostic value of fibrinogen in patients with coronary artery disease and prediabetes or diabetes following percutaneous coronary intervention: 5-year findings from a large cohort study. Cardiovasc Diabetol. 2021;20(1):143.
22. Neergaard-Petersen S, Hvas AM, Kristensen SD, Grove EL, Larsen SB, Phoenix F, Kurdee Z, Grant PJ, Ajjan RA. The influence of type 2 diabetes on fibrin clot properties in patients with coronary artery disease. Thromb Haemost. 2014; 112(6):1142–1150.
23. Al-Barjas HS, Ariens R, Grant P, Scott JA. Raised plasma fibrinogen concentration in patients with abdominal aortic aneurysm. Angiology. 2006; 57(5):607–614.
24. Snoep JD, Hovens MM, Eikenboom JC, van der Bom JG, Huisman MV. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. Arch Intern Med. 2007; 167(15):1593–1599.
25. Watala C, Pluta J, Golanski J, Rozalski M, Czyz M, Trojanowski Z, Drzewoski J. Increased protein glycation in diabetes mellitus is associated with decreased aspirin-mediated protein acetylation and reduced sensitivity of blood platelets to aspirin. J Mol Med (Berl). 2005; 83(2):148–158.
26. Hess K, Alzahrani SH, Mathai M, Schroeder V, Carter AM, Howell G, Koko T, Strachan MWJ, Price JF, Smith KA, et al. A novel mechanism for hypofibrinolysis in diabetes: the role of complement C3. Diabetologia. 2012;55(4):1103–13.
27. Ajjan RA, Gamlen T, Standeven KF, Mughal S, Hess K, Smith KA, Dunn EJ, Anwar MM, Rabbani N, Thornalley PJ, et al. Diabetes is associated with posttranslational modifications in plasminogen resulting in reduced plasmin generation and enzyme-specific activity. Blood. 2013;122(1):134–42.
28. Kearney K, Tomlinson D, Smith K, Ajjan R. Hypofibrinolysis in diabetes: a therapeutic target for the reduction of cardiovascular risk. Cardiovasc Diabetol. 2017; 16(1):34.
29. Gerbaud E, Darier R, Montaudon M, Beauvieux MC, Coffin-Boutreux C, Coste P, Douard H, Ouattara A, Catargi B. Glycemic variability is a powerful independent predictive factor of midterm major adverse cardiac events in patients with diabetes with acute coronary syndrome. Diabetes Care. 2019;42(4):674–81.
30. Parker WAE, Storey RF. Aspirin dosing in atherosclerotic cardiovascular disease: should we be more ADAPTABLE? Cardiovasc Res. 2021; in press.
31. Jones WS, Mulder H, Wruck LM, Pencina MJ, Kripalani S, Muñoz D, Crenshaw DL, Effron MB, Re RN, Gupta K, et al. Comparative effectiveness of aspirin dosing in cardiovascular disease. N Engl J Med. 2021;384:1981.
32. Tehrani S, Antovic A, Mobarrez F, Mageed K, Lins PE, Adamson U, Wallen HN, Jorneskog G. High-dose aspirin is required to influence plasma fibrin network structure in patients with type 1 diabetes. Diabetes Care. 2012; 35(2):404–408.
33. Steg PG, Bhatt DL, Simon T, Fox K, Mehta SR, Harrington RA, Held C, Andersson M, Himmelmann A, Ridderstrale W, et al. Ticagrelor in patients with stable coronary disease and diabetes. N Engl J Med. 2019;381(14):1309–20.
34. Bhatt DL, Bonaca MP, Bansilal S, Angiolillo DJ, Cohen M, Storey RF, Im K, Murphy SA, Held P, Braunwald E, et al. Reduction in ischemic events with ticagrelor in diabetic patients with prior myocardial infarction in PEGASUS-TIMI 54. J Am Coll Cardiol. 2016;67(23):2732–40.
35. Eikelboom JW, Connolly SJ, Bosch J, Dagenais GR, Hart RG, Shestakovska O, Diaz R, Alings M, Lonn EM, Anand SS, et al. Rivaroxaban with or without aspirin in stable cardiovascular disease. N Engl J Med. 2017;377(14):1319–30.

36. Spectre G, Arnetz L, Ostenson CG, Brismar K, Li N, Hjemdahl P. Twice daily dosing of aspirin improves platelet inhibition in whole blood in patients with type 2 diabetes mellitus and micro- or macrovascular complications. Thromb Haemost. 2011; 106(3):491–499.
37. Parker WAE, Storey RF. Novel approaches to P2Y12 inhibition and aspirin dosing. Platelets. 2020; 32(1):7–14.
38. Parker WAE, Orme RC, Hanson J, Stokes HM, Bridge CM, Shaw PA, Sumaya W, Thorneycroft K, Petrucci G, Porro B, et al. Very-low-dose twice-daily aspirin maintains platelet inhibition and improves haemostasis during dual-antiplatelet therapy for acute coronary syndrome. Platelets. 2019;30(2):148–57.

Publisher's Note