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REGULAR ARTICLE

The effects of direct current cardioversion for persistent atrial fibrillation on indices of endothelial damage/dysfunction

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Abstract

Background: Atrial fibrillation is associated with increased thromboembolic risk, and this risk may occur even following cardioversion. Atrial fibrillation has been hypothesised to cause alterations in endothelial cell function through the influences of altered flow dynamics, and resultant endothelial dysfunction may be contributory to the generation of a prothrombotic state. The aim of this study was therefore to assess endothelial function before and after electrical cardioversion.
Methods: We studied 30 consecutive patients undergoing elective cardioversion for AF and compared them with 20 healthy controls. Plasma levels of endothelial damage/dysfunction [von Willebrand factor (vWF), E-selectin (E-sel), soluble thrombomodulin (sTM)] and Circulating Endothelial Cells (CECs, an index of endothelial damage) in whole blood were measured in all subjects and on the AF group at baseline (pre-cardioversion) and at 2 h and 4 weeks following cardioversion.
Results: Plasma levels of vWf were significantly increased in persistent AF at baseline compared to healthy controls ($p<0.001$). With restoration of sinus rhythm, vWF levels were significantly decreased at 4 weeks ($p=0.0001$), whilst levels of CECs ($p=0.01$) and sTM ($p=0.022$), although not increased at baseline, were significantly increased following cardioversion.
Conclusion: Although plasma vWF levels decreased post-cardioversion, suggesting some improvement in vascular endothelial function, the increases in sTM and CECs at 4 weeks may indicate endothelial injury sustained peri-cardioversion. This (delayed)

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injury and shedding of endothelial cells post-cardioversion may contribute to late thromboembolic risk.

Introduction

Atrial fibrillation (AF) is associated with an increased risk of stroke and thromboembolism [1], but the precise mechanism(s) for this remains unclear, although a prothrombotic or hypercoagulable state may contribute.

Endothelial damage/dysfunction is implicated as the initial pathophysiological process in thrombogenesis and atherogenesis and is associated with inflammation, impairment of vasodilatation and hypercoagulability. Endothelial function may be demonstrated in vivo by measurement of plasma markers originating from the endothelium, and by functional measurements such as plethysmography or flow-mediated dilatation [2]. Circulating endothelial cells (CECs) have also been demonstrated in conditions associated with endothelial injury, and are thought to be evidence of direct vessel wall damage [3—7].

Increases in inflammatory markers [8], impairment of forearm vasodilatation [9] and a hypercoagulable state [10], especially with regard to plasma levels of von Willebrand factor (vWf, an index of endothelial damage/dysfunction [11—15]), have all been demonstrated in AF, indicating a role for the endothelium in the pathophysiology of hypercoagulablity in AF. Other endothelial indices, including soluble thrombomodulin (sTM; an indicator of endothelial damage), and E-selectin (E-sel; an indicator of endothelial cell activation) are also abnormal in AF in comparison to sinus rhythm controls [16—18]. Further evidence for endothelial damage comes from scanning electron microscopy data [19], as well as immunohistochemistry studies showing increased vWf and tissue factor (TF) expression in atrial tissue from patients with AF [20,21]. Certainly, it has been hypothesised that alterations in haemodynamic forces with the onset of AF could adversely influence the function of the endothelium, and in vitro studies with cultured endothelial cells, have shown that changes in flow conditions can be associated with alterations in both nitric oxide (NO) production and adhesion molecule expression [22,23]. However, we are unaware of previous studies examining CECs, nor the effects of cardioversion on endothelial indices, in patients with AF.

We hypothesised that numbers of CECs may be raised in non-valvular AF, alongside vWF, E-sel and sTM as endothelial plasma markers of dysfunction, activation and damage, respectively, and that there would be a change in endothelial markers and in the number of CECs following restoration of sinus rhythm by electrical cardioversion. To investigate this further, we studied a cohort of consecutive patients with persistent AF undergoing cardioversion in our unit.

Patients and methods

Over a period of 6 months, we recruited 30 eligible consecutive patients with persistent AF (duration >3 weeks, but <12 months since AF first documented electrocardiographically), undergoing elective DC cardioversion in our unit. All patients were fully anticoagulated with warfarin (target INR 2.0—3.0) for a minimum of 3 weeks pre-cardioversion, and were continued on therapeutic anticoagulation beyond follow-up. In keeping with standard practice in our unit, all patients were treated with beta-blockers (or if not tolerated, amiodarone 200 mg/day) pre- and post-cardioversion. Exclusion criteria were known acute causes of AF (such as thyrotoxicosis, pneumonia or other infections, alcohol excess, pulmonary embolism), significant heart valve disease or left ventricular dysfunction (classified as anything more than mild on echocardiography), acute cardiovascular or cerebrovascular events (myocardial infarction, acute coronary syndromes or stroke) within 3 months, malignancy, connective tissue disease, chronic infection, inflammatory conditions, renal or hepatic dysfunction.

These AF patients underwent synchronised direct current cardioversion under brief general anaesthesia (Propafol), using a monophasic defibrillator with paddles placed in anterior—lateral positions (up to 360 J in a 200 J, 200 J, 360 J sequence).

Baseline results in AF patients were compared with age- and sex-matched 'healthy controls' recruited from hospital staff and preoperative clinics for minor procedures, including hernia repairs, cataract surgery, etc. All healthy controls were 'healthy' by virtue of careful clinical history and examination, as well as basic blood screening tests, an echocardiogram and an electrocardiogram. These subjects are included to provide a perspective (i.e. what should be 'healthy' levels) for the patients' data. No direct case—control comparison

is intended. The study had the approval of the local research and ethics committee, and all participants gave written informed consent.

Laboratory methods

From a single venepuncture, venous blood was collected first for routine laboratory analysis, then into separate vacutainers containing sodium citrate and sodium fluoride from fasted AF subjects on the morning of cardioversion and at 2 h then at 4 weeks post procedure, and from healthy controls at the screening visit. Citrated samples were put directly on ice and fluoridated samples kept at room temperature, and all bloods were processed within 20 min of collection. Platelet-poor citrated plasma was obtained from venous blood by centrifugation at 3000 rpm for 20 min at 4 °C, and aliquots were stored at −70 °C to allow batch analysis. Soluble E-selectin (ng/ml) was measured by ELISA with R&D Systems reagents (Abingdon, United Kingdom). vWf (iU/dl) was measured by an established ELISA (Dako, Ely, UK). Soluble thrombomodulin (ng/ml) was measured by ELISA (kits from Diagnostica, Stago, France). The intra-assay coefficient of assays was <5% and inter-assay variation was <10%.

Blood for CECs, collected in a sodium fluoride tube, was prepared for immunomagnetic separation. The detailed methods for capturing CECs and criteria for counting CECs in our unit has been previously described [4], and is based on an original methodology by Mutin et al. [3]. Intra- ($n=40$) and inter-assay ($n=20$) coefficients of variation were <5% and <10%, respectively. Slides were counted by a single observer under epifluorescence microscopy (Zeiss) at a later date. The inter- and intra-observer variations of the method in our laboratory were <5%. All laboratory work was performed blinded to patient identity.

Statistical analysis

Results are expressed as mean ± standard deviation (± S.D.) or median and interquartile range (IQR). Baseline data between patients and controls was analysed by chi-squared tests for categorical data and by *t*-testing or Mann—Whitney test as appropriate for parametric and nonparametric continuous variables, with Tukey's post hoc analysis as appropriate. Sequential data pre- and post-cardioversion were analysed by Friedman's Repeated Measures Analysis of Variance. Correlations were performed by Spearman's rank correlation method. Stepwise multiple regression analyses were performed to determine independent predictors for plasma vWF, E-sel, sTM and CEC, using age, sex, smoking status, body mass index, systolic and diastolic blood pressure, total cholesterol levels, as well as the presence of cardiovascular co-morbidities and presence of AF. All statistical analysis was performed using Minitab version 13 and SPSS version 11. A *p*-value of <0.05 was considered statistically significant.

Results

We studied 30 consecutive patients with persistent AF undergoing DC cardioversion (23 male, mean age 64 ± 10 years). The mean duration of documented AF pre-cardioversion was 6.1 ± 3.3 months. These sub-

Table 1 Demographic data and results for AF patients versus controls

	Controls	AF	*P* value
N	20	30	—
Demographic and clinical data			
Age (years)	63.4 ± 8.9	63.6 ± 9.6	0.935
Sex (male/female)	12:8	23:7	0.368
Smokers, *n* (%)	1 (5%)	1 (3%)	0.768
Systolic BP (mm Hg)	129 ± 17	136 ± 19	0.208
Diastolic BP (mm Hg)	76 ± 8	82 ± 14	0.107
Total cholesterol (mmol/dl)	5.7 ± 1.0	4.4 ± 1	<0.001
Body mass index	26 ± 4	28 ± 6	0.096
Comorbidities			
Treated hypertension	0	14 (46%)	—
Treated hyperlipidaemia	0	3 (10%)	—
Diabetes mellitus	0	1 (3%)	—
IHD	0	5 (17%)	—
CVA	0	3 (10%)	—
Results of baseline research indices			
vWF (iU/dl)	100 ± 51	169 ± 41	<0.001
E-sel (ng/ml)	28 (12—55)	48 (24—86)	0.1373
sTM (ng/l)	36 (25—49)	43 (21—56)	0.6559
CEC (cells/ml)	4.5 (1.6—7.2)	5.3 (3.7—9.3)	0.1687

BP = blood pressure; IHD = prior history of ischaemic heart disease (i.e. angina or myocardial infarction); CVA = prior cerebrovascular accident (i.e. stroke or transient ischaemic attack); vWF = von Willebrand factor; E-sel = soluble E-selectin; sTM = soluble thrombomodulin; CEC = circulating endothelial cells.

Indices are expressed as mean ± standard deviation or median and interquartile range, with *t*-test or Mann—Whitney used for statistical analysis as appropriate.

jects were compared with a group of 20 age- and sex-matched healthy controls. Cholesterol levels were significantly lower in the AF patients than age- and sex-matched healthy controls, but groups were well matched in other baseline characteristics (Table 1). AF patients had significantly higher levels of vWF ($p<0.0001$), but not E-sel, sTM, or CECs when compared to healthy controls at baseline.

Of the 30 consecutive patients undergoing cardioversion using a monophasic defibrillator, only 15 (50%) patients were successfully cardioverted to sinus rhythm. At 4 weeks follow-up (median time to follow up 35.5 days, IQR 30—37), 13 patients remained in sinus rhythm and 17 patients were in AF, and on this basis, the patients were sub-grouped for subsequent analysis.

Effect of cardioversion

For patients with AF converted to sinus rhythm (Table 2), vWF levels were significantly decreased and median sTM and CEC levels were significantly increased at 4 weeks following cardioversion (Friedman's repeated measures ANOVA $p=0.021$ for vWF, $p=0.001$ for sTM, $p=0.033$ for CECs).

Levels of sTM and CECs were also raised in those patients who underwent the procedure but remained in AF at follow-up but this did not reach statistical significance ($p=0.232$ and $p=0.327$, respectively); there is a small drop in vWF at 2 h, which then returned to baseline levels ($p=0.039$ for change). There were no significant changes in E-selectin levels following cardioversion in either group.

Table 2 Follow-up data for atrial fibrillation patients maintaining sinus rhythm and those remaining in AF after cardioversion

	Patients in sinus rhythm ($n=13$)	Patients in AF ($n=17$)
Age (years)	66 (62.5—67.5)	67 (61—69)
Sex (male/female)	12:1	11:6
Body mass index	25 ± 4	30 ± 6
Systolic BP (mm Hg)	136 ± 19	136 ± 20
Diastolic BP (mm Hg)	78 ± 15	84 ± 13
Cholesterol (mmol/dl)	4.3 ± 1.2	4.5 ± 1.0
Baseline vWF (iU/dl)	185 ± 33	156 ± 43
2-h vWF (iU/dl)	157 ± 50	135 ± 44
4-week vWF (iU/dl)	130 ± 48*	157 ± 43
Baseline E-sel (ng/ml)	48 (28—86.5)	56 (20—107)
2-h E-sel (ng/ml)	40 (29—60)	36.5 (24—57)
4-week E-sel (ng/ml)	42 (28.5—55)	48 (25—60)
Baseline sTM (ng/ml)	44 (8—64)	42.5 (26—55)
2-h sTM (ng/ml)	60 (51—80)	62.5 (45—85)
4-week sTM (ng/ml)	72.5 (55—82.5)**	70 (25—92.5)
Baseline CEC (cells/ml)	5 (3—6)	5.5 (4.4—11.)
2-h CEC (cells/ml)	5 (2—15)	7 (4—13)
4-week CEC (cells/ml)	16.5 (5—27.5)†	11 (8—19)

vWF = von Willebrand factor; E-sel = soluble E-selectin; sTM = soluble thrombomodulin; CEC = circulating endothelial cells.

Indices are expressed as mean ± standard deviation or median and interquartile range (IQR), with *t*-test or Mann—Whitney used for statistical analysis as appropriate.

Using Friedman's repeated measures ANOVA *$p=0.021$ for vWF, **$p=0.001$ for sTM, †$p=0.033$ for CECs, for those achieving and maintaining sinus rhythm at 4 weeks. These levels did not significantly change for those remaining in AF.

Univariate and multiple step-wise regression analysis

For the whole group, on univariate analysis, vWF levels were predicted not only by the presence of AF (Spearman, $r=0.594$, $p<0.0001$), but also by systolic blood pressure ($r=0.445$, $p=0.002$). vWF levels also significantly correlated with CECs ($r=0.355$, $p=0.029$).

There were no significant correlations between vWF and sTM or E-sel levels, and no relationships between vWF levels and a history of previous cardiovascular disease or treated risk factors (i.e. ischaemic heart disease, a cerebrovascular event, diabetes mellitus, hypertension or hyperlipidaemia) (data not shown). On stepwise multiple regression analysis, the only independent predictor of baseline plasma vWF levels was the presence of AF ($p<0.0001$).

Discussion

The thromboembolic risk associated with AF is well documented, but disappointingly, the presumed benefits of restoration of sinus rhythm in patients with persistent AF have not translated into clinical benefits in terms of outcome in cardioversion arms of the AFFIRM, RACE and HOT CAFE studies ([24—26], for review, see [27]). These trials have been criticised because they do not in their analysis allow for the high relapse rate and failure to cardiovert AF, but further evidence is still needed to determine the pathophysiological effects of successful cardioversion on AF patients.

Studies up until now have shown some differences in atrial endocrine function, platelet activation, and coagulation parameters following cardioversion [22—31]. This study is consistent with previous observations on plasma indices of endothelial abnormalities in patients with persistent AF, as com-

pared with healthy controls [15]. We acknowledge, of course, that the presence of other cardiovascular disease and risk factors in our AF population may independently cause endothelial damage/dysfunction, but this does not explain the observed reduction in vWF to near-healthy control levels following restoration of sinus rhythm. Patients were also maintained on the same medication pre- and post-cardioversion, whether successful or not, so no changes in medications or anticoagulation status should have impacted on our results. In common with Nikitovic et al. [32], we found a significant decrease in vWF levels in those patients maintaining sinus rhythm at 4 weeks, in contrast to the group of AF patients who failed cardioversion, (in whom vWF levels at 4 weeks did not alter significantly). We acknowledge that a previous study in our department failed to show a change in vWF levels following cardioversion, but this may have been due to relatively low pre-cardioversion vWF levels at baseline in the persistent AF group [15].

Raised vWF levels are widely accepted as an indicator of endothelial damage/dysfunction in AF and this has been consistent across numerous studies. Our findings, in this study, of a reduction in vWF with the restoration of sinus rhythm, may therefore be some indication of restoration of overall endothelial function. However, the unexpected findings of increased levels of CECs and sTM levels at 4 weeks following cardioversion would indicate endothelial damage, at a time at which endothelial function should be recovering, if there were indeed a return to normal flow dynamics after successful treatment of AF. Indeed, CEC have been described up until now in conditions that have in common the presence of vascular injury, although small numbers are found circulating in the peripheral blood of normal adults [3—7]. In this study, and our previous studies [4,33], CECs were defined using an endothelial specific antigen CD146 which is almost exclusively expressed on mature endothelial cells. In our hands [33], CECs are almost entirely eNOS-staining positive (suggesting that CECs are mural-endothelial in origin), but effectively CD31 (PECAM-1), CD34 (endothelial progenitor cells, EPC), CD36 (microvascular cells) and CD45 (leukocyte common antigen) *negative*. Indeed, recent data would suggest that these CECs do originate from vessel walls [34], and would therefore in our study indicate that there may be endothelial injury following cardioversion.

sTM is of course a key co-factor in the initiation of the protein C anticoagulant pathway that is active as an integral endothelial cell membrane protein (for review, see [35]). Raised levels not only have been traditionally seen as an indicator of actual endothelial cell membrane damage, but it has also been demonstrated that sTM has an important regulatory role in inflammation and that recombinant TM-like molecules in vitro can protect cultured endothelial cells from cell death [36]. This could provide an explanation for the raised sTM levels found in AF patients post-cardioversion and is one possible explanation as to why vWF levels were not raised in association with increased sTM in our study.

sE-sel, used as an indicator of endothelial activation, is again not significantly raised in AF, nor are levels significantly altered by electrical cardioversion, suggesting that the endothelium perhaps is damaged rather than expressing molecules associated with activation in AF.

Whilst our original hypothesis was that electrical cardioversion itself may be damaging to the endothelium in the acute phase, we find that following restoration of sinus rhythm a gradual improvement in endothelial function may occur as evidenced by decreasing vWF levels. However, this appears to be an over-simplification, as our findings would suggest a more complex endothelial response, with perhaps *delayed* rather than immediate endothelial cell shedding following electrical cardioversion of AF. Indeed, the raised CECs may indicate endothelial injury and a delayed detachment, rather than the direct endothelial damage presumed to occur in the other conditions in which CECs have been shown to be raised. Although the *precise* time course of this endothelial cell injury, and possible release of procoagulant factors or inflammatory cell contents, is uncertain, this could be a possible mechanism for late thromboembolic risk in AF patients who are cardioverted or undergo attempted DC cardioversion, as evident from recent large randomised trials [27]. Further studies would be warranted in this area both to determine the origin of increased circulating endothelial cells this late post-cardioversion, and to examine their role in the pathophysiology of thrombogenesis.

Limitations

This study is obviously limited by small numbers and a low successful cardioversion rate. Due to strict exclusion criteria of co-morbidities (except *treated* cardiovascular risk factors) that would significantly alter endothelial function, the number of patients undergoing cardioversion in a 6-month period that could be included in our study was restricted. For this study, we defined successful electrical cardioversion as return to sinus rhythm after DC cardioversion with maintenance of sinus rhythm at 2 h,

and the groups were analysed on the basis of their maintenance of sinus rhythm at 4 week follow-up, therefore any patients with early recurrence of AF were not deemed to have a successful cardioversion. Our low successful cardioversion rate could have also been impacted on by the use of a monophasic rather then a biphasic defibrillator, the relatively long duration of AF and low use of concomitant amiodarone.

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