Increased left atrial thrombin generation in mitral stenosis is not reflected in arterial prothrombin fragment 1 + 2 levels

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ABSTRACT

A proportion of patients with mitral stenosis have increased left atrial thrombin generation, with elevated left atrial but normal peripheral venous levels of prothrombin fragment 1 + 2 (F1 + 2). Whether this pattern of left atrial and venous F1 + 2 levels is related to limited spillover of F1 + 2 from the left atrium into the systemic circulation, or to washout of increased left atrial F1 + 2 production into the arterial circulation with subsequent systemic clearance, is unclear. We examined the relationship between arterial and venous F1 + 2 levels in mitral stenosis patients without left atrial thrombus. The study group comprised 36 patients with either a normal (n = 29) or prolonged (n = 7) international normalized ratio (INR; a measure of clotting time) who were undergoing percutaneous balloon mitral valvuloplasty. Baseline arterial and venous blood samples were collected at the beginning of the valvuloplasty procedure, and left atrial and venous samples were collected after trans-septal puncture. The left atrial F1 + 2 level exceeded the corresponding venous level in patients with a normal INR (P < 0.03); however, baseline arterial and venous F1 + 2 levels were similar. Arterial and venous F1 + 2 levels were also similar in the subgroup of patients with evidence of a regional increase in left atrial thrombin generation, and were not different from arterial and venous F1 + 2 levels in patients without such an increase. Baseline arterial and venous F1 + 2 levels were both lower in the presence of a prolonged INR. Thus the pattern of increased left atrial but normal venous F1 + 2 levels in mitral stenosis is due to limited spillover from the left atrium into the systemic circulation.

INTRODUCTION

Left atrial thrombus is a serious and common complication of mitral stenosis [1–3]. To increase understanding of the pathogenesis of this complication, studies have focused on the assessment of left atrial coagulation activity in blood samples obtained during percutaneous balloon mitral valvuloplasty [4–7]. An important observation that has emerged from such studies is that, in the absence of left atrial thrombus, a proportion of mitral stenosis patients have elevated left atrial levels, but normal peripheral venous levels, of the coagulation marker prothrombin fragment 1 + 2 (F1 + 2), a peptide byproduct of the conversion of prothrombin into thrombin [4,5,7]. While this finding suggests that patients with mitral stenosis are prone to the development of a hypercoagulable state, characterized by a regional increase in left atrial thrombin generation, it remains unclear whether the lack of an associated elevation of peripheral venous F1 + 2 levels [4] is due to limited spillover of F1 + 2 from the left atrium into the systemic circulation or to washout of increased left atrial F1 + 2 production.

Key words: atrium, coagulation, mitral stenosis.
Abbreviations: F1 + 2, prothrombin fragment 1 + 2, INR, international normalized ratio.
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into the arterial circulation, with subsequent clearance by systemic tissues.

The distinction between these two mechanisms has potentially important clinical implications, because washout of increased left atrial F1 + 2 production into the arterial circulation might provide a potential means of diagnosing increased left atrial coagulation activity with the use of combined peripheral arterial and venous blood sampling. Evidence that arterial F1 + 2 levels may be elevated in mitral stenosis has been presented as part of a brief report by Yamamoto et al. [7], but the significance of this observation was limited by two factors. First, most patients were anticoagulated with warfarin, a drug which decreases coagulation activity [8,9], and it is thus unclear to what extent arterial F1 + 2 levels are altered in the presence of normal clotting times. Secondly, the presence of only 12 patients in the study group precluded comparison of arterial and venous F1 + 2 levels in the presence and absence of increased left atrial thrombin generation. Accordingly, the aims of the present study were (1) to compare arterial and venous levels of F1 + 2 in mitral stenosis patients with normal clotting times, (2) to examine and compare the relationship between arterial and venous F1 + 2 levels in mitral stenosis patients with and without increased left atrial thrombin generation, and (3) to compare the relationship between arterial and venous levels of F1 + 2 in patients with a prolonged or normal international normalized ratio (INR; a measure of clotting time).

METHODS

Subjects

The study group comprised 36 adult patients (seven men; 29 women) aged 50 ± 12 years (mean ± S. D.) with mitral stenosis (New York Heart Association class II or III) who underwent percutaneous balloon mitral valvuloplasty at our institution between February 1993 and November 1996. Valvuloplasty was not performed in the presence of greater than grade 2/4 mitral regurgitation, significant coronary artery disease, other significant valvular heart disease or left atrial thrombus diagnosed at the time of transoesophageal echocardiography. In addition, all patients in the study group had a plasma D-dimer level (a marker of fibrinolysis) of < 200 ng/ml at the time of valvuloplasty, a value representing the upper limit of normal in the absence of left atrial thrombus [10]. No patient had a history of renal or liver disease, malignancy, deep venous thrombosis or pulmonary embolism, and none of the women were pregnant. The study was conducted in accordance with guidelines established by the National Health and Medical Research Council of Australia, and was approved by the Monash Medical Centre Human Research and Ethics Committee. Written informed consent was obtained from all subjects before blood sampling.

Twenty four patients in the study group had been on continuous full-dose warfarin therapy for > 3 months before the mitral valvuloplasty because of a previous thromboembolic episode or a recognized risk factor for left atrial thrombus formation. As described previously [4,5], warfarin therapy was withdrawn from these patients on admission to hospital 4 days before the valvuloplasty procedure, and an intravenous infusion of sodium heparin was then commenced at a dose which maintained the activated partial thromboplastin time at 2–3 times the upper limit of the normal range. The sodium heparin infusion was stopped at least 4 h before patient transfer to the catheterization laboratory, and the activated partial thromboplastin time was confirmed to be within the normal range at the beginning of the valvuloplasty procedure. Concurrently, patients were subdivided into groups with normal or prolonged clotting times, based on whether their INR was < 1.2 (n = 29) or ≥ 1.2 (n = 7) respectively [5].

Echocardiography

Prior to valvuloplasty, transthoracic echocardiography was performed with a 2.5 MHz transducer and a Hewlett Packard phased array Sonos 1000 system to assess left atrial diameter using standard M-mode criteria [11]. Transoesophageal echocardiography was also performed before valvuloplasty with a Hewlett Packard Omniplane transducer, and adequate views of the left atrial cavity and appendage were obtained in all patients, not only to exclude left atrial thrombus but also to assess for the presence of left atrial spontaneous echo contrast, an independent predictor of increased left atrial thrombin generation [4]. Left atrial thrombus was diagnosed by the presence of a clearly defined intracavity mass, acoustically distinct from the underlying endocardium [12]. Left atrial spontaneous echo contrast was diagnosed on the basis of dynamic smoke-like echoes within the left atrial cavity and appendage that had a characteristic swirling motion distinct from white noise artifact [13].

Experimental protocol

At the beginning of the valvuloplasty procedure, baseline blood samples were collected from the femoral arterial and venous sheaths, and a bolus dose of 2500 units of sodium heparin was then given intravenously. Following atrial trans-septal puncture with a Brockenborough needle, an 8F trans-septal catheter was advanced into the body of the left atrium. Blood samples were subsequently collected from the left atrium and the femoral venous sheath, approx. 60 min after baseline blood sampling.

After discarding the initial 5 ml of blood, 4 ml of blood was collected from each sampling site into a sterile syringe and transferred immediately to an evacuated
polyethylene terephthalate tube containing 3.13% sodium citrate (Vacuette, Greiner, Germany). Blood samples were centrifuged within 2 h of collection at 2500 g and 4 °C for 10 min to obtain platelet-poor plasma, which was then separated and stored at −70 °C until assay.

Assay procedure
Commercial enzyme immunoassays were used to measure plasma levels of F1 + 2 (Enzygnost prothrombin fragment 1 + 2; Behringwerke AG, Marburg, Germany) and D-dimer (Dimertest EIA; Agen Biomedical Ltd, Brisbane, Australia). In our laboratory, intra-assay and interassay variability for F1 + 2 is 6.2% and 9.5% respectively, and intra-assay and interassay variability for D-dimer is 6.0% and 9.0% respectively. The activated partial thromboplastin time was measured using Actin FSL Activated PTT Reagent (Dade-Behring, Marburg, Germany). The INR was measured using a recombinant tissue factor (Dade-Behring). The International Sensitivity Index was calculated for each batch to standardize the INR. The normal range of INR in our laboratory is < 1.2.

Data analysis
Results are expressed as means ± S.D., and were analysed using standard statistical tests. Within-patient comparisons of baseline femoral arterial and venous, and of post-trans-septal left atrial and venous, coagulation marker levels were performed by paired t test if normally distributed, or the Wilcoxon signed rank test if not normally distributed. Coagulation marker level data from the same site in different patient groups were compared using an unpaired t test if normally distributed, or the Mann–Whitney test if not normally distributed. A P value of < 0.05 was considered significant.

RESULTS
In patients with a normal INR, left atrial and venous levels of D-dimer were not different (85 ± 32 and 80 ± 29 ng/ml respectively; P = 0.59), but the left atrial level of F1 + 2 exceeded the corresponding venous level (1.23 ± 0.75 and 0.92 ± 0.36 nmol/l respectively; P < 0.002). However, baseline arterial and venous levels of F1 + 2 were similar (0.95 ± 0.50 and 0.91 ± 0.41 nmol/l respectively; P = 0.52; Figure 1), despite the evidence for an increase in left atrial thrombin generation.

Effect of left atrial thrombin generation on systemic F1 + 2 levels
Of the patients with a normal INR, a subgroup with increased left atrial thrombin generation was defined on the basis of a left atrial F1 + 2 level greater than the upper 95% prediction interval of the regression equation representing the normal variability associated with measurement of peripheral venous F1 + 2 levels in our laboratory [4]. No difference was evident in clinical, echocardiographic or haemodynamic variables between patients with increased and normal left atrial thrombin generation (Table 1). Furthermore, baseline arterial and venous F1 + 2 levels were similar in patients with normal (P = 0.58; Figure 2A) and increased (P = 0.49; Figure 2B) left atrial thrombin generation. Moreover, baseline venous (P = 0.58) and arterial (P = 0.35) F1 + 2 levels did not differ between patients with normal and increased left atrial thrombin generation.

Table 1 Clinical and haemodynamic characteristics and F1 + 2 levels in mitral stenosis patients with normal or increased left atrial thrombin generation

<table>
<thead>
<tr>
<th></th>
<th>Normal left atrial generation</th>
<th>Increased left atrial generation</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>49 ± 12</td>
<td>53 ± 6</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>5 (26%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Venous F1 + 2 (nmol/l)</td>
<td>0.90 ± 0.40</td>
<td>0.98 ± 0.29</td>
</tr>
<tr>
<td>Left atrial F1 + 2 (nmol/l)</td>
<td>0.93 ± 0.45</td>
<td>1.81 ± 0.88</td>
</tr>
<tr>
<td>Left atrial spontaneous echo contrast</td>
<td>15 (79%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>Left atrial diameter (cm)</td>
<td>5.3 ± 0.7</td>
<td>5.7 ± 1.0</td>
</tr>
<tr>
<td>Cardiac index (litres·min⁻¹·m⁻²)</td>
<td>2.19 ± 0.52</td>
<td>2.44 ± 0.44</td>
</tr>
<tr>
<td>Mitral valve area (cm²)</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Mitral valve mean gradient (mmHg)</td>
<td>13 ± 5</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>Left atrial pressure (mmHg)</td>
<td>22 ± 6</td>
<td>21 ± 7</td>
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</table>

Figure 1 F1 + 2 levels in femoral vein and femoral artery blood samples from mitral stenosis patients with a normal INR
NS, not significant.
Effect of prolonged INR on systemic F1 + 2 levels

In patients with a prolonged INR, left atrial and venous D-dimer levels were similar (92 ± 38 and 86 ± 35 ng/ml respectively; P = 0.50). As previously reported [5], left atrial and venous levels of F1 + 2 were also not different, but both left atrial (P < 0.03) and venous (P < 0.01) F1 + 2 levels were lower than in patients with a normal INR. Baseline arterial and venous F1 + 2 levels were similar in the presence of a prolonged INR (0.48 ± 0.19 and 0.44 ± 0.08 nmol/l respectively; P = 0.58; Figure 3), and both arterial (P < 0.01) and venous (P < 0.01) baseline F1 + 2 levels were lower compared with those in patients with a normal INR.

DISCUSSION

In this study we have examined the relationship between femoral arterial and venous levels of F1 + 2, a marker of thrombin generation, in mitral stenosis patients without left atrial thrombus. Specifically, we sought to determine whether the normal venous F1 + 2 levels observed in patients with increased left atrial thrombin generation [4] are due to limited spillover of F1 + 2 from the left atrium or to release of the elevated left atrial F1 + 2 into the arterial circulation with subsequent systemic clearance. Our findings indicate that arterial and venous levels of F1 + 2 are similar in the setting of either a normal or prolonged INR, even in a subgroup of patients with a clear-cut increase in regional left atrial thrombin generation. These findings suggest that the association of increased left atrial with normal venous F1 + 2 levels in mitral stenosis is due principally to limited spillover of F1 + 2 from the left atrium into the systemic circulation. An important corollary of this conclusion is that comparison of F1 + 2 levels in peripheral arterial and venous blood samples in mitral stenosis patients is unlikely to be useful for the clinical detection of increased left atrial thrombin generation.

Our conclusion that there is limited spillover of F1 + 2 from the left atrium into the systemic circulation in mitral...
stenosis is unlikely to have been influenced by our experimental design. Thus, although coagulation markers such as fibrinopeptide A and thrombin–antithrombin complex are prone to undergo artefactual increases during blood sampling through catheters [14,15], thrombin generation in the present study was assessed by measurement of F1+2, a coagulation marker with a low propensity for sampling-related elevations [15,16]. Indeed, we have shown previously that there is no difference between levels of F1+2 collected via needle puncture and introducer sheath or introducer sheath and long catheter in mitral stenosis patients [4]. To minimize the effects of factors such as heparin administration and trans-septal puncture, comparison of arterial and venous F1+2 levels in the present study was performed at the beginning of the valvuloplasty procedure. On the other hand, the extent of regional left atrial thrombin generation was assessed by comparing left atrial and venous F1+2 levels measured after trans-septal puncture. Given the similarity of the baseline and post-trans-septal venous F1+2 levels (P > 0.3; results not shown), however, it is unlikely that any substantial changes in thrombin generation occurred between these two time points.

In our previous study, minor residual INR prolongation following warfarin cessation in mitral stenosis patients was accompanied by lower venous and left atrial F1+2 levels than were present in patients with a normal INR [5]. Moreover, in the same study, the left-atrial–venous F1+2 difference was also lower in patients with a prolonged INR, consistent with a preferential suppression of left atrial thrombin generation by warfarin [5]. On the basis of these results, it is therefore not surprising that arterial F1+2 levels in the present study were reduced in patients with a prolonged INR, because this would be expected irrespective of whether arterial F1+2 levels were more closely related to left atrial or venous levels.

Our finding that arterial levels of F1+2 did not exceed venous levels in mitral stenosis is clearly at variance with the study of Yamamoto et al. [7], who reported that levels of F1+2 were significantly higher in the artery than the vein. A comparison of the experimental designs of the two studies provides no ready explanation for this discrepancy. Thus both study groups were undergoing percutaneous mitral valvuloplasty for symptomatic mitral stenosis, and the groups were similar with respect to age, left atrial pressure, mitral gradient and mitral valve area. Furthermore, left atrial thrombus was excluded by transoesophageal echocardiography, and levels of D-dimer were within the normal range and similar in both study groups.

However, two aspects of the study of Yamamoto et al. [7] are difficult to understand on the basis of existing literature. Firstly, full-dose warfarin was used up to the day prior to valvuloplasty in 10 out of 12 patients, resulting in a mean INR of 2.1 immediately before the valvuloplasty procedure. Given that this degree of INR prolongation is known to markedly suppress F1+2 levels [9,16–21] and our previous report that even minor prolongation of the INR is associated with both lower venous and left atrial F1+2 levels and an attenuated left-atrial–venous F1+2 difference [5], it is surprising that Yamamoto et al. [7] found not only peripheral venous levels of F1+2 in the range found in patients with a normal INR [4,16], but also a more than 2-fold difference between left atrial and peripheral venous F1+2 levels. Secondly, a comparison of femoral venous (0.85 nmol/l) and femoral artery (2.09 nmol/l) F1+2 levels in the study of Yamamoto et al. [7] indicates that >50% clearance of this coagulation marker occurred within its first passage across the lower limb. Although no studies have been performed on the systemic kinetics of F1+2 in mitral stenosis patients, this observation is clearly at odds with data from Bauer et al. [22], who administered 131I-F1+2 intravenously into normal patients and found that ~90 min was required to achieve a 50% decrease in venous plasma levels of the radiolabelled F1+2.

In summary, the results of the present study indicate that increased left atrial thrombin generation in mitral stenosis without left atrial thrombus is not accompanied by elevated arterial levels of F1+2, consistent with the notion that in this condition there is limited spillover of this coagulation marker from the left atrium into the systemic circulation.

ACKNOWLEDGMENTS

R.E.P. was supported by a Postgraduate Medical Research Scholarship from the National Heart Foundation of Australia.

REFERENCES


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Received 4 August 1999/19 October 1999; accepted 13 January 2000