A circadian variation exists for soluble levels of intercellular adhesion molecule-1 and E-selectin in healthy volunteers

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INTRODUCTION

For many disease states there is a daily cycle of disease activity. It has been well documented that there is a peak incidence of cerebrovascular [1] and cardiovascular [2] events in the early hours of the morning. This peak seems to match an early morning dip in fibrinolytic activity [3], a rise in blood viscosity [4] and platelet aggregation [5], all of which predispose to thrombosis. Previously we have shown a circadian variation in leucocyte activation [6] and endothelial function. [7]. This may be important in the pathophysiology of thrombotic events but also in inflammatory disorders, which also show a circadian variation in disease activity. Patients with rheumatoid arthritis have increased symptoms of joint stiffness and discomfort in the morning, which eases off throughout the day [8,9].

Cell adhesion molecules (CAMs), which are present on the surface membrane of many cells including leucocytes and endothelial cells, are important in the trafficking of leucocytes from the circulation into the tissues [10]. Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin family of CAMs and is found on endothelial cells and leucocytes. E-selectin is only expressed on cytokine-activated endothelial cells. Recently, soluble forms of these molecules have been identified in the blood [11]. These molecules are released from the bound form after stimulation by inflammatory mediators and may be markers for endothelial and leucocyte activation. They have been identified in healthy volunteers [11], with elevated levels in certain inflammatory [12,13] and neoplastic disease states [14].

The aim of this study was to identify any circadian variation in soluble cell adhesion molecule (sCAM) levels, which may be related to the diurnal variation in disease activity or occurrence of certain disorders, in addition to potentially showing a need for standardization of time points for sampling in clinical studies.

SUBJECTS AND METHODS

Subjects

This study was performed on normal volunteers who agreed to spend a 24 h period in Ninewells Hospital and Medical School. The study was approved by the local Medical Ethics Committee. Ten male subjects who gave informed consent were recruited from laboratory and medical staff. They had a median age of 24 (range 17–33) years, all were non-smokers and none had taken any medication in the previous 14 days. During the study, subjects were asked to carry out their usual daily activities but to refrain from rigorous activities. They remained ambulant until 00:10 h; thereafter they were asked to retire to bed and remain recumbent until 08:10 h. During the 24 h
of the study, subjects ate as self-selected diet according to the schedule shown in Table 1.

### Blood sampling and sICAM-1 and sE-Selectin assays

Ten millilitres of blood were drawn at each time point. Each sample was obtained from a different site within the antecubital fossa without the use of venostasis and using a 19-gauge butterfly needle. The samples were allowed to coagulate at 37 °C for 1 h before being centrifuged and stored at -70 °C until analysis.

Serum levels of sICAM-1 and sE-selectin were measured using commercially available sandwich-linked immunosorbent assay (ELISA) kits (R&D Systems, U.K.). In brief, specific antibody conjugated to horseradish peroxidase was added to murine anti-human sICAM-1 or sE-selectin antibody-coated microtitre ELISA plates. Standards and diluted samples were then added to the plates, which were covered and incubated for 1.5 h at room temperature. After washing, substrate (tetramethylbenzidine) was added to each well, the plate again covered and incubated. After 30 min, the stop solution (sodium azide) was added and the absorbance of each well was determined using a spectrophotometer. The results were calculated from a standard curve.

### Statistical analysis

The results were analysed by repeated measures analysis of variance looking for any statistically significant circadian variation in sICAM-1 and sE-selectin levels allowing for inter-subject differences. Significance values refer to the P values obtained after operating multivariate analysis of variance for all time points and subjects. The null hypothesis was rejected at P < 0.05. All analyses were performed using the statistical package INSTAT (GraphPAD Software, CA, U.S.A.).

### RESULTS

The results are given in Figure 1. There is a statistically significant circadian variation in both sE-selectin and sICAM-1 levels (P < 0.0001). Soluble ICAM-1 had a maximum value at 12:00 h [238.3 (17.1) ng/ml; mean (S.E.M.)], falling to a minimum at 04:00 h [203.6 (13.8) ng/ml]. Soluble E-selectin levels also reached a peak at 12:00 [53.88 (8.8) ng/ml], but fell to a trough at 00:00 h [48.14 (7.3) ng/ml]. Serum levels of sE-selectin and sICAM-1 measured by other workers [15] from controlled subjects were 53 ± 17 ng/ml and 283 ± 19 ng/ml respectively. These are very similar to our own results and show that our assay methodology is producing expected results.

### DISCUSSION

Cell adhesion molecules are important in promoting the inflammatory response and, by allowing the inflammatory cell access to infected tissues, attenuating infection. These CAMs may also be implicated in pathological inflammation [16] and may have a role in many inflammatory diseases, for example rheumatoid arthritis [12,13]. They may also be linked to the metastasis of neoplastic cells [14].

There are three families of CAMs. E-selectin is a member of the selectin family. It is present only on the surface of endothelial cells activated by inflammatory mediators such as interleukin-1 and tumour necrosis factor-α. Its function is to promote the rolling of the leucocyte along the surface of the endothelium within the blood vessels [17]. This then allows tighter bonds to form between other CAMs such as members of the integrin and immunoglobulin families. ICAM-1 is one member of the immunoglobulin family of CAMs and can be found on the surface membrane of endothelial cells and leucocytes [10]. Recently, circulating or soluble isoforms have been identified in the blood [11]. Evidence suggests that these molecules may be cleaved or released from the bound forms [18], perhaps by proteolytic cleavage. The mechanism responsible for their release, however, is not fully understood nor is their function. However, it has been shown that sE-selectin retains its ability to bind to leucocytes, activates the integrin CD11b/CD18 (mac-1) [19] and also acts as a chemoattractant to leucocytes [20]. These functions only occur at concentrations greater than...
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would be expected to be found in the circulation. However, the necessary concentrations may be attained locally at sites of inflammation. Similarly, a biological function has been shown for sICAM-1. This soluble molecule has been shown to be a receptor for the rhinovirus [21] and also plasmodium-infected erythrocytes [22]. It too retains its ability to bind to its ligand on leucocytes [23]. This may be important in promoting the de-adhesion of leucocytes from the endothelium, thus allowing their trans-endothelial migration.

Soluble E-selectin and sICAM-1 molecules have been identified in the blood in healthy subjects with elevated levels being found in certain disease states [24]. Soluble E-selectin levels are elevated in patients with multiple sclerosis [25], vasculitis [26] and in septic shock [27]. In the latter, levels seemed to correlate inversely with disease outcome. Since E-selectin is produced by and expressed solely on activated endothelial cells, the presence of its soluble form in the circulation may be a good indication of endothelial cell activation. The low levels of E-selectin identified in healthy subjects may relate to the small inflammatory and vascular insults the body is subjected to throughout the day. Soluble ICAM-1 can also be measured in plasma in healthy subjects. Elevated levels have been shown to occur in many inflammatory disorders including systemic sclerosis [28], systemic lupus erythematosus [29] and Wegener's granulomatosis [30].

It has also been identified in infections such as malaria [31] and in patients with malignancies [14]. Levels of sICAM-1 have been found to correlate with other markers of inflammatory disease activity, for example the erythrocyte sedimentation rate.

No one has clearly described the expected sCAM half-life in blood. The sCAMs are released into the plasma by proteolytic cleavage [24]. In vitro cytokine stimulation of endothelial cells produces E-selectin expression after 4 h, which returns to baseline 12 h later. ICAM-1 expression occurs after 24 h and remains elevated for 96 h [32]. It is interesting to speculate as to whether all the CAMs such as vascular cell adhesion molecules (vCAM) exhibit a circadian variation. We have previously shown such a rhythm to exist in healthy volunteers for sP-selectin, the platelet cell adhesion molecule, which totally mirrors the circadian fluctuation seen in platelet count [33].

In conclusion, we have shown, for the first time, that a circadian variation in sICAM-1 and sE-selectin levels exists in healthy volunteers. This further confirms the circadian variation in endothelial cell and leucocyte activation which we [6,7,34] and others [8] have previously shown. It may help to explain why some symptoms of inflammatory and thrombotic conditions are maximal in the early morning. It may be of interest to postulate that this rhythm may be altered in inflammatory diseases, as we have previously shown in thrombotic disease [35]. Further studies are underway. In addition, the findings from this study identify the need to standardize the time of blood sampling in future studies where these molecules are to be assessed.

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REFERENCES


