The adhesion molecule P-selectin and cardiovascular disease

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The adhesion molecule P-selectin (CD62P) is of interest because of its role in modulating interactions between blood cells and the endothelium, and also because of the possible use of the soluble form as a plasma predictor of adverse cardiovascular events. Although present on the external cell surface of both activated endothelium and activated platelets, it now seems clear that most, if not all, of the measured plasma P-selectin is of platelet origin. P-selectin is partially responsible for the adhesion of certain leukocytes and platelets to the endothelium. Animal models have also shown the important role of P-selectin in the process of atherogenesis. For example, increased P-selectin expression has been demonstrated on active atherosclerotic plaques; in contrast, fibrotic inactive plaques lack P-selectin expression, and animals lacking P-selectin have a decreased tendency to form atherosclerotic plaques. Increased levels of soluble P-selectin in the plasma have also been demonstrated in a variety of cardiovascular disorders, including coronary artery disease, hypertension and atrial fibrillation, with some relationship to prognosis. The objective of this review is to provide an overview of the current literature on this molecule and thus present a concise view of its potential in dissecting the pathophysiology of atherosclerosis. In doing so we shall focus primarily on human biology but will note a small number of excellent lessons provided by non-human work.

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Introduction
Changes in platelet and endothelial cell function are apparent in atherosclerosis. Endothelial injury and/or activation, by whatever cause, leads to the production of certain factors and, indeed, changes to the endothelium membrane, a consequence of which is direct and targeted contact with platelets and leukocytes.\(^1\) This concept has therefore led to considerable interest in the mechanisms of platelet and leukocyte interactions, then to the discovery of many adhesion molecules, such as intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), and the selectin family of molecules (P-selectin, E-selectin and L-selectin) that together play important roles in the initiation of leukocyte migration into the vascular wall.\(^2\) A variant of these adhesion molecules can also be detected in the plasma (hence soluble adhesion molecules), leading to the presumption that they are secreted, shed or cleaved from the cell membrane as part of the disease process. However, the evidence that changes in soluble levels in the plasma accurately reflect levels at the cell membrane is slim.

Among these cell adhesion molecules, P-selectin is of interest because of its expression, under defined conditions, by both platelets and endothelial cells. The aim of this review is to provide an overview of this molecule and its role in different cardiovascular disease processes. We will first consider its role(s) at the cell membrane,
subsequently examining physiological and pathological aspects of the molecule in plasma.

Structure, function and cell expression of P-selectin

P-selectin (CD62P), the largest of the selectins, with a mass of 140 kDa, extends approximately 40 nm from the endothelial surface (Fig. 1). Previous names include granule membrane protein 140 (GMP-140) and platelet activation dependent granule external membrane protein (PADGEM).² It is a component of the membrane of the alpha and dense granules of platelets, and also of the membrane of the Weibel–Palade bodies of endothelial cells. In common with the other selectins, P-selectin has an N-terminal lectin domain, an epidermal growth factor motif, (generally) nine regulatory protein repeats, a transmembrane section and a short intracytoplasmic tail.³ Binding of anti P-selectin monoclonal antibodies to stimulated endothelial cells in vitro induces increased intracellular calcium (mimicking events that follow polymorphonuclear leukocyte adherence), implying a possible signalling role⁴ although this remains unconfirmed.

Expression on endothelial cells

Within minutes of stimulation of endothelial cells in vitro by inflammatory mediators, such as histamine, thrombin, or phorbol esters, or hypoxia, Weibel–Palade bodies are mobilized and degranulate their von Willebrand factor. P-selectin is also expressed at the surface as rapidly as two minutes after stimulation. However, this expression can be short lived, reaching its peak after only 10 min, declining to baseline after 3 h.⁵–⁷ Additional synthesis of P-selectin is brought about within 2 h by cytokines, such as interleukin-1, tumour necrosis factor-α, and by thrombin, lipo polysaccharide or oxygen radicals.⁵–⁸ Similarly, upon incubation of platelets with agents such as adenosine or epinephrine, there is increased P-selectin expression at the surface of the platelet. Since P-selectin is a component of the membrane of platelet alpha and dense granules, and as degranulation is widely believed to be synonymous with activation, it therefore follows that increased expression of this molecule at the platelet surface reflects activation⁹-¹⁰ Of further interest is the report that nitric oxide (NO) is a regulator of P-selectin expression as inhibitors of NO synthase increased P-selectin expression.¹¹ This may be clinically important as Minamino et al.¹² suggest that concurrent low NO metabolites and high platelet P-selectin expression are linked.

Although P-selectin will bind to heparan sulphate and fucoidan, its primary ligand is P-selectin glycoprotein ligand-1 (PSGL-1), a dimeric molecule rich in O- and N-glycans, that is constitutively expressed on almost all leukocytes (Fig. 2). Cloned by Sako et al.,¹³ its structure and functions have now been described in detail.¹⁴-¹⁹ PSGL-1 is found on a number of haemopoietic cells such as neutrophils, lymphocytes, eosinophils, monocytes and other myeloid progenitor cells where it mediates tethering and adhesion ¹.⁴,²⁰,²¹ However, it is to some extent non-specific as PSGL-1 also acts as a ligand for the other selectins, and there are approximately 25 000 molecules on each leukocyte.²² The cell and molecular biology of PSGL-1 has recently been the subject of excellent reviews.¹⁷,²³

Numerous in vivo and in vitro experiments in (knock-out) mice and humans have clearly illustrated the role of P-selectin (and other adhesion molecules) and PSGL-1 in supporting platelet-leukocyte interactions and in leukocyte rolling on the endothelium.²⁴-²⁹ This may include signalling events within the endothelium.⁴ Indeed, in mice deficient for P-selectin, leukocyte rolling is defective and may also involve L-selectin.³⁰-³⁴ In TNF-α stimulated vessels, P-selectin and E-selectin tend to have overlapping functions.³⁴ In mice deficient for P-selectin, it is necessary to block E-selectin function to significantly reduce rolling, and in E-selectin knockouts, an antibody against P-selectin must be introduced to reduce rolling. Correspondingly, no leukocyte rolling is observed in E-selectin/P-selectin double deficient mice treated with
TNF-α. Observations of rolling flux fraction and rolling velocities indicate that P-selectin is responsible for early rolling while E selectin allows slow rolling and more adhesion.31,35 The density of P-selectin on an activated endothelium in vivo is unknown (but see below) — however, immunohistochemical studies suggest that it is much lower than that of E selectin.17 Numerous excellent reviews on the mechanism of selectin/ICAM/VCAM-mediated tethering, adhesion, rolling and extravasation are available.36,37

Immunohistochemical analysis of surgically-excised and post-mortem human atherosclerotic plaques has shown strong expression of P-selectin by the endothelium overlying active atherosclerotic plaques and fatty streaks. This pattern of staining demonstrated a strong (P<0.001) correlation with the expression of ICAM-1. P-selectin was not, however, detected in normal arterial endothelium or in endothelium overlying inactive fibrous plaques.38 Adhesion of monocytes and related cell lines to these tissues were inhibited by an antibody directed towards P-selectin that correlated with the specific endothelial localization of this adhesion molecule.39 In a baboon model, P-selectin expression increased after focal brain ischaemia and reperfusion.40 Tenaglia et al.41 reported significantly greater P-selectin staining on atherectomy specimens from patients with unstable angina than from patients with stable angina, although there was no difference in the expression of E selectin. These experiments support the concept that the increased expression of P-selectin could be important in atherosclerosis.

**Expression on platelets**

As P-selectin was originally defined on (activated) platelets, and as they are easier to obtain and hence study, there are, unsurprisingly, considerably more platelet than endothelial data.42,43 There are approximately 10 000 P-selectin molecules on the surface of an activated platelet, translating to a density of perhaps 350 sites/µm², a density that exceeds that on even a thrombin or histamine stimulated endothelial cell in vitro by an approximate factor of ten.17

Perhaps the greatest (clinical) use to which this molecule has so far been put is as a marker of platelet activation. Previous to this, plasma markers (such as beta thromboglobulin and platelet factor 4) and physical activity (e.g. in aggregation) had been used.10 However, the ease of flow cytometry has put this technique in the foreground, and can be used to define the presence of various molecules on the platelet surface, such as P-selectin, CD63, the gpib/V/IX complex, gpIV, gpVI, gpIB/IIla, and neo-antigens such as PAC-1.44,45 Some of these molecules are expressed constitutively, but as P-selectin is expressed only on activated cells,42,45 it has achieved popularity in defining this population in conditions such as atrial fibrillation,12 diabetes and hypertension,46 congestive heart failure,47 stroke48 and in acute coronary syndromes.49,50 Increased expression of P-selectin (and CP63, a component of the lysosomal granule membrane) by platelets from patients with type II hypercholesterolaemia was reduced by 8-weeks treatment with fluvastatin.51
P-selectin expression on activated platelets appears not to be simply to aid leukocyte and/or endothelial adhesion: there is evidence that it is also important for inter-platelet aggregation, stabilizing the initial gpllb/IIIa–fibrinogen interactions, thus allowing the formation of large and stable platelet aggregates. Merten et al.\textsuperscript{52} showed that inhibition with monoclonal antibodies to P-selectin was able to achieve about 95 to 100\% of de-aggregation, whilst antibodies to PSGL-1, gplb or gpllb/IIIa had no effects. This further shows that P-selectin causes this aggregation by receptors other than PSGL-1. Further differences between P-selectin and gpllb/IIIa include a role for the former in pulsatile (as opposed to non-pulsatile) shear-induced aggregation, a condition more likely to be present in the environs of stenotic or atheromatous arteries.\textsuperscript{53}

In addition to the above-mentioned properties of P-selectin in facilitating the adhesion of platelets and neutrophils to the endothelium, it has also been suggested that P-selectin is responsible for further activating the endothelium and setting up a positive feedback mechanism. Indeed, P-selectin has additional procoagulant activities in that it also regulates the production of platelet activating factor by monocytes and appears to prime monocytes for increased phagocytosis,\textsuperscript{54} as well as inducing the formation of tissue factor\textsuperscript{55,56} — thus possibly having a role in inflammation and atherogenesis.

The creation of P-selectin deficient knock-out mice has, as indicated, provided many interesting insights. Perhaps unsurprisingly, such mice demonstrate a prolonged bleeding time and increased haemorrhagic lesions.\textsuperscript{57} However, more interesting is the report that atheromatous lesions induced in P-selectin deficient mice were larger and were more calcified if normal platelets were transfused.\textsuperscript{58} Furthermore, P-selectin null hearts transplanted into wild-type mice demonstrated marked reduction in graft neutrophil infiltration (n.b. not merely adhesion), and also prolonged graft survival.\textsuperscript{5} Leaving aside the cross-species caveat, these are, perhaps, more convincing lines of evidence of the importance of this molecule in atherothrombotic disease. The close interplay between P-selectin, platelets, leukocytes and atherosclerosis is summarized in Fig. 3.

**Soluble P-selectin in plasma**

**Origin and specificity**

The development of monoclonal-antibody based ELISAs has allowed the quantification of P-selectin in fluids. The first commonly-asked question is: exactly what is being measured? This point has arisen from the discovery of messenger RNA/cDNA coding for different variants of P-selectin, some of which code for a molecule lacking the transmembrane portion, implying direct ‘secretion’ from the cell.\textsuperscript{3,59} It follows that the increased presence of a truncated soluble P-selectin isoform in plasma may reflect increase in the activity of this form of message.\textsuperscript{60} Alternative mechanisms for the appearance of soluble P-selectin include simple shedding, or active cleavage from cell surface, presumably by a non-specific enzyme or other mediator(s) that may arise from leukocytes, the endothelium, or elsewhere.\textsuperscript{61} Platelet derived soluble P-selectin and plasma P-selectin have both been shown to react with antibodies against the cytoplasmic domain,
implying at least some soluble P-selectin may arise from damaged platelet membranes. Aged purified platelets, destined for transfusion, continue to lose P-selectin and release beta-thromboglobulin ex-vivo during storage, suggesting at least one passive process.

The second question often asked is: where does the P-selectin come from? As P-selectin is present within both endothelial cells and the platelets, there has been considerable debate whether or not raised plasma levels of P-selectin reflect endothelial dysfunction, platelet activation, or both. Semenov et al. (reporting strong correlations between soluble P-selectin and platelet count) and Fijnheer et al. suggest that under normal conditions, the majority of the P-selectin is from platelets, supporting our concurrent hypothesis and data. However, Fijnheer et al. also concluded that endothelial cell activation is associated with an increased P-selectin concentration per platelet. This position is also supported by other evidence, such as the lack of correlation between soluble P-selectin and the gold standard plasma endothelial marker, von Willebrand factor, but a better correlation with established specific platelet product, beta-thromboglobulin. Also crucial is the failure of endothelial stimulant desmopressin to increase soluble P-selectin in vivo despite increases in plasma von Willebrand factor. Cell immunoassays indicate that, relative to total cell protein, less P-selectin is present in human umbilical vein endothelial cells (HUVECs) than in platelets and although we have been unable to find any P-selectin in HUVEC lysates, this may be due to poor ELISA sensitivity. Semenov et al. reported P-selectin in endothelial cultures and supernatants, but Jilma et al., like us, were unable to find P-selectin in HUVEC supernatants.

Further clinical evidence pointing to the platelet origin of P-selectin are reports of increased plasma levels of this marker in platelet consumption disorders, such as thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome. In these disorders, there is actual platelet destruction or consumption as part of the disease process. Chong et al. further demonstrated a correlation between P-selectin levels and beta-thromboglobulin (a marker of platelet activation). These findings strongly imply a platelet origin for P-selectin.

Study of megakaryocytes and P-selectin are also an important tool in resolving this puzzle of the origin of circulating plasma P-selectin. Platelets have a limited capacity to produce substances de novo, although they do have messenger RNA. Most of the substances that are found in mature circulating platelets are preformed in the megakaryocyte. Indeed, P-selectin is known to regulate megakaryocytopoiesis. For example, Jilma et al. have provided interesting evidence for the platelet origin of plasma P-selectin, studying levels in patients with bone marrow aplasia prior to bone marrow or stem cell transplantation, finding a significant decrease in the levels of P-selectin with time. After platelet transfusion, the levels of P-selectin dramatically rose in these patients, providing a clear indication of the platelet origin of P-selectin.

Overall, we conclude that, in the 5 years since our original hypothesis, there are little serious data to contradict the position that raised soluble P-selectin reflects platelet disturbance. Indeed, almost all the data we have available, and others to come, are confirmatory of this point.

Cross sectional studies in overt cardiovascular disease

The first predominately clinical papers focussing on soluble P-selectin in atherothrombotic disease began to appear some 10 years ago. Wu et al., described raised levels 1 day after acute myocardial infarction that peaked on day 3, and also in the acute phase of cerebrovascular thrombosis. Although Katayama et al. were cautious in their interpretation, both Chong et al. and Wu et al. clearly indicated that soluble P-selectin could be a useful marker of thrombosis.

Various groups have reported raised soluble P-selectin in stable peripheral artery disease and stable coronary artery disease (but no correlation with von Willebrand factor, tissue plasminogen activator or plasminogen activator inhibitor), hypertension (again, with no von Willebrand factor correlation), unstable angina (but not in stable angina, although this group consisted of only 11 patients), increased levels after intra-coronary injection of acetyl-choline in patients with angina, and serially following acute myocardial infarction. We have shown raised soluble P-selectin levels in atherosclerosis but this was more related to disease of the ilio-femoral arteries (and not of carotid disease), with increased levels in more widespread disease.

In cerebrovascular disease, Frijns et al. found raised levels in patients with symptomatic internal carotid artery stenosis, and further increases in acute ischaemic stroke. However, this group, largely on the basis of a highly significant, albeit not very strong (r=0.36, P=0.044) correlation with soluble E selectin, concluded that increased P-selectin reflects activation of both endothelial cells and platelets. Concurrently, the same group concluded that platelets are the major source of circulating P-selectin in healthy individuals, and reported a very strong correlation between soluble P-selectin and platelet count (r=0.91), as have others. Bath et al. reported soluble P-selectin in different sub-types of acute ischaemic stroke: levels were higher in total anterior circulation infarct compared to lacunar infarct – however, von Willebrand factor failed to differentiate these types of stroke, suggesting a platelet, as opposed to vascular, aetiology.

Subsequent work by numerous groups has confirmed raised soluble P-selectin in a wide variety of acute and chronic cardiovascular conditions.

Cross sectional studies in the risk factors for cardiovascular disease

Raised soluble P-selectin has been reported in diabetes (where it failed to correlate with von Willebrand factor, smoking and hypertension). Raised levels
have also been noted in hypercholesterolaemia, with both strong\textsuperscript{101} and absent\textsuperscript{92} correlations with endothelial marker von Willebrand factor, although the latter group reported a correlation with beta thromboglobulin. Others failed to find a difference in soluble P-selectin in hypercholesterolaemia, with no correlation to von Willebrand factor.\textsuperscript{93} Parissis et al. noted that normocholesterolaemic hypertensives had lower soluble P-selectin than hypercholesterolaemia hypertensives, but higher levels than normotensive controls.\textsuperscript{94} Osterud et al.,\textsuperscript{95} studying 266 healthy subjects, reported raised levels in female compared to female non-smokers (but not in male smokers versus non-smokers), higher levels in men compared to women, no effect of age, and a positive correlation with cholesterol. In a smaller population of 186 healthy subjects, we also found no effect of age, but could not confirm the previous report of the effect of gender.\textsuperscript{96} We conclude, therefore, that evidence of raised soluble P-selectin in the risk factors for atherosclerosis is not as strong as that in overt disease.

The effects of pharmacological intervention

Riondino et al.\textsuperscript{97} found that normalization of blood pressure in elderly (mean age 74 years) hypertensives (mean SBP/DBP 186/103 mm Hg) with an ACE-inhibitor and/or a calcium antagonist resulted in a reduction in platelet aggregation and both the cell expression and plasma levels of P-selectin. Nomura\textsuperscript{46} treated 23 diabetic hypertensives with a calcium channel inhibitor for 8 weeks, and found a reduction in soluble P-selectin of 6\% and a reduction in soluble E selectin of 13\% (both $P<0.05$); the latter probably reflecting improved endothelial function with lower blood pressure.

Statins are a group of lipid-lowering drugs that are now being recognized as having effects beyond cholesterol reduction alone, such as effects on inflammation and on the endothelium. However the data on platelet function per se are limited. For example, Pucetti et al.\textsuperscript{98} demonstrated a significant improvement in platelet function as evaluated by plasma markers, that is, lowering in P-selectin levels with different statins (including simvastatin, fluvastatin, pravastatin, and atorvastatin) in patients with hypercholesterolaemia. Statins have also been shown to reduce the levels of P-selectin post acute coronary syndrome,\textsuperscript{99} and in patients with stable coronary heart disease.\textsuperscript{100} Others have reported that lipid-lowering with statins does reduce soluble P-selectin in either the presence or absence of atherosclerosis.\textsuperscript{101–103} One possible mechanism may be that statins act by stimulating production of endothelial nitric oxide, which then decreases platelet activation.\textsuperscript{102}

A 6-month combined package of optimum medical care, consisting of anti-hypertensive and lipid-lowering therapies, alongside continuing advice to cut back on/stop smoking, was effective in reducing blood pressure, total cholesterol, von Willebrand factor and soluble P-selectin in 53 high-risk (i.e. also with, for example, hypercholesterolaemia, diabetes or smoking) hypertensives.\textsuperscript{105} However, others have failed to find any change in soluble P-selectin in 50 hypercholesterolaemic patients (including several diabetics and several with hypertension) after 3 months successful treatment with a statin (pravastatin).\textsuperscript{104} Interestingly, however, the same group demonstrated a significant improvement in endothelial function. Similarly, pravastatin failed to reduce soluble P-selectin, but did reduce von Willebrand factor, in 17 patients with atherosclerosis and borderline (mean total cholesterol 6.5 mmol/l) hypercholesterolaemia after 4 months treatment.\textsuperscript{105}

Percutaneous revascularization interventions

Tsakiris et al.\textsuperscript{106} tested the effect of peripheral angioplasty on soluble P-selectin and other molecules in 71 patients with peripheral atherosclerosis. This cohort included 25 diabetics, who had higher soluble P-selectin than non-diabetics. Only 1 h after the procedure, there was a reduction in soluble P-selectin and soluble E selectin but not in von Willebrand factor; but after 6 months, the reduction in soluble P-selectin remained low and the soluble E selectin level returned to pre-angioplasty levels. This data may be interpreted as a brief relief from persistent platelet and endothelial cell activation by the improved blood flow, but that only platelet function benefits in the long term, and is consistent with the view of a large burden of atheroma within the peripheral circulation.\textsuperscript{90} Although levels before intervention were not statistically different in those 30 with restenosis at 6 months, versus the 41 without restenosis, soluble P-selectin was indeed higher in the restenosis group.

Ishiwata et al.\textsuperscript{107} performed a similar experiment, but of coronary angioplasty on 73 subjects, and at 6 months classified them into those with and without restenosis. There was no change in soluble P-selectin in the subjects free of restenosis, but levels increased after the intervention by 24\% in those whose lesions restenosed. They also found a significant correlation between soluble P-selectin and beta thromboglobulin. It is tempting to speculate that raised soluble P-selectin in both groups whose disease returned was related to excess and continuing platelet activity.

Other interventions

Stopping smoking results in a reduction in soluble P-selectin,\textsuperscript{89} but the reverse (i.e. observing the effect of acute smoking of two cigarettes in quick succession) failed to increase levels.\textsuperscript{108} Andrew et al.\textsuperscript{109} noted no change in soluble P-selectin in 20 young (mean age 26 years) diabetics before and after a 3-month course of 1000 IU vitamin E daily. However, Davi et al.\textsuperscript{91} dosed 20 hypercholesterolaemic patients with 600 mg vitamin E daily for 2 weeks, and observed a reduction in soluble P-selectin (by 40\%), von Willebrand factor (by 13\%), and urinary 11-dehydroxy thromboxane B$_2$ (by 49\%). Nomura et al.\textsuperscript{46} treated 17 diabetics with a platelet aggregation inhibitor for 4 weeks, reporting a strong ($P<0.001$) reduction in soluble (by 49\%) and platelet-membrane (by 53\%) P-selectin and a small (by 13\%, $P<0.05$) reduction in...
endothelial marker soluble thrombomodulin. In both these reports, the greater reduction in soluble P-selectin compared to the endothelial marker, suggests to us that this is due to improved platelet function, and that the improved endothelial function is a secondary consequence.

Two groups have measured P-selectin before and after methionine loading to induce an acute hyperhomocysteinaemia. One\(^{109}\) found no change in soluble P-selectin (but a rise in von Willebrand factor), whilst the other\(^{111}\) reported no change in P-selectin in older (aged 55–70 years) subjects, but a decrease in younger (age 21–40 years) subjects. More data on the effects of hyperhomocysteinaemia on platelet function are awaited.

Antithrombotics, anticoagulants and thrombolysis

If P-selectin, either soluble or platelet-expressed, is indeed a platelet product, then we may therefore expect an influence by anti-platelet and/or anti-thrombotic therapy. Aspirin seems unable to alter soluble P-selectin levels at rest or after induction with lipopolysaccharide or exercise.\(^{112–114}\) However, a definite effect on membrane P-selectin is not as clear. Moshfegh et al.\(^{115}\) found that clopidogrel, with or without aspirin, suppressed P-selectin expression, as did Malinin et al.\(^{116}\) with aspirin alone. However, Serebrauny et al. reported raised levels compared to controls despite 7 days of aspirin use by patients with coronary artery disease\(^{117}\) and Michelson et al. failed to find an effect on surface P-selectin although it did (as expected) reduce plasma thromboxane.\(^{118}\) Ten Berg et al.\(^{119}\) reported lower membrane P-selectin in 26 patients taking aspirin plus a coumarin compared to 26 matched patients taking aspirin alone, whilst Kamath et al.\(^{120}\) found, in a cross-sectional study, no difference in soluble P-selectin in 34 patients with atrial fibrillation (AF) not on therapy compared to 30 on aspirin and 58 on Warfarin. However, the same group\(^{121}\) also reported that soluble P-selectin increased by 24% in 35 patients with AF 6 weeks after starting on warfarin with a concurrent fall in beta thromboglobulin. This effect was not seen in 35 other AF patients after 6 weeks of aspirin and clopidogrel.

Knight et al.\(^{122}\) concluded that increased expression of membrane P-selectin following coronary stenting was due to heparin, possibly acting via thromboxanes and prostacyclins. Although this may initially seem important as it implies that heparin activates platelets, this anticoagulant can inhibit P-selectin interactions in vitro.\(^{123}\) Amin et al.\(^{124}\) suggested that raised soluble P-selectin in patients with heparin-induced thrombocytopenia might be due to the anticoagulant, the underlying disease, or both. Subsequent falls in soluble P-selectin followed the withdrawal of this drug and the use of the anti-thrombin argatroban.

Two groups\(^{50,125}\) have shown increased soluble P-selectin three hours after therapeutic thrombolysis for acute myocardial infarction, and one of these\(^{50}\) also reported no difference in membrane bound P-selectin. This does not necessarily imply that thrombolysis is directly activating platelets as the increase may reflect the infarction itself, or perhaps that the streptokinase/reteplase/alteplase is physically digesting the molecule from the surface of the platelet.

A reduction in membrane expression of P-selectin with aspirin use fits in well with the marker reflecting platelet activation, although no influence on soluble levels is a puzzle. Overall, however, we suggest that no clear-cut conclusion can yet be made on P-selectin in relation to anticoagulant and antithrombotic therapies.

Follow-up studies of soluble P-selectin in cardiovascular disease

In population-based studies increased levels of soluble P-selectin in citrated plasma have been shown to predict major cardiovascular events in patients with existing peripheral or coronary atherosclerosis\(^{126,127}\) and in apparently healthy women.\(^{128}\) However, these studies failed to provide comparator molecules. Increased levels of soluble P-selectin in hypertension\(^{46,76,81}\) appear to be unable to predict adverse cardiovascular events, although von Willebrand factor and D-dimers can do so.\(^{129}\) Hollander et al. assessed the value of soluble and membrane P-selectin in identifying patients with acute coronary syndromes,\(^{130}\) concluding that, based on sensitive and specificity, neither has any advantage over the MB isoform of creatinine kinase (CK). However, Hillis et al.\(^{131}\) concluded that both soluble P-selectin and troponin I, but not soluble E selectin, ICAM, VCAM or CK-MB, were independent predictors of the 38 from 126 patients with chest pain thought clinically to represent myocardial ischaemia who subsequently experienced a serious cardiac event.

Two studies been unable to find any additional value in the measurement of soluble P-selectin. Mulvihill et al.\(^{132}\) found that raised soluble VCAM and C-reactive protein, but not soluble ICAM, E selectin or P-selectin, were able to predict which 27 from 91 patients with unstable angina and myocardial infarction would go on to suffer a major cardiovascular end point. Malik et al.,\(^{133}\) performing a 16-year follow-up of 643 men with coronary artery disease and 1278 controls, found that soluble P-selectin was unlikely to add much predictive information to that provided by more established risk factors. However, as both these studies measured soluble P-selectin in serum, it seems unlikely that comparisons to the other studies, who used citrated plasma, can be drawn. As it is clear that, as discussed, the greater part, if not all, soluble P-selectin in the blood arises from platelets, then some unknown proportion of levels in serum may well reflect a contribution from platelets involved in clotting,\(^{134,135}\) as Malik et al. themselves recognized.\(^{133}\)

Thus, as yet, there are only relatively small and uncontrolled (by a comparator molecule) studies of the ability of soluble P-selectin to predict major cardiovascular end points. Additional head-to-head data from large (>1000 subjects?) studies are awaited.
Future directions

P-selectin as a therapeutic target

As discussed, P-selectin and its ligand PSGL-1 mediate cell–cell adhesion, and platelet /endothelial/neutrophil interactions/cross-talk are a good model of this process.\textsuperscript{25,136–139} Unsurprisingly, therefore, monoclonal antibodies directed towards P-selectin, or soluble P-selectin itself, blocks these in vitro interactions.\textsuperscript{25,139–141} These studies, in turn, have lead to in vivo experiments where various combinations of P-selectin and PSGL-1 analogues, and antibodies to these molecules, were used to ameliorate various animal models of cardiovascular disease.\textsuperscript{26,141–145} However, as yet, no substantial reports of the use of these agents in humans are available.

Whole platelet P-selectin

Platelet activation, defined by degranulation, leads to the appearance of P-selectin at the surface.\textsuperscript{142,146} However, in vivo, this (murine) post-activation expression of P-selectin may be short-lived and lost/shed into the plasma.\textsuperscript{147} However, in a similar experiment, Michelson et al. have also shown (although in the baboon) that circulating degranulated platelets rapidly lose surface P-selectin, and that levels in the plasma pool rise, but, crucially, also that these P-selectin negative platelets continue to circulate and function.\textsuperscript{148} This latter experiment has implications for the value of using membrane bound P-selectin as a totally reliable marker of activation as, for example, it is unclear whether or not this phenomenon occurs in humans, or what the timescale of shedding can be. An alternative to membrane and soluble P-selectin is to simply measure the total mass of P-selectin in a detergent lysate of a given number of platelets (say, \(10^8\)), thus providing the index of the mass of P-selectin per platelet. Using this technique, we found a lower absolute mass of P-selectin per platelet (\(101\times10^{-6}\) ng/cell) in untreated patients with atrial fibrillation when compared to healthy controls (\(180\times10^{-6}\) ng/cell).\textsuperscript{149} The effect of aspirin was (seemingly) to reduce this amount (to \(43\times10^{-6}\) ng/cell), whilst the effect of Warfarin was (also seemingly) to increase this amount (to \(225\times10^{-6}\) ng/cell). The significance of these preliminary data, and its relationship with soluble P-selectin, are unclear but additional studies are progressing. Circulating activated platelets may also be detected using a whole platelet ELISA.\textsuperscript{150}

Genetics

Early work on the structure of the P-selectin gene (clustered with the E and L selectin genes on 1q21–q24) found it to be highly polymorphic, with the reduced frequency of a certain allele in patients with myocardial infarction.\textsuperscript{151,152} Subsequent work has identified other haplotypes associated with an increased risk of myocardial infarction.\textsuperscript{153} These studies, although most promising, require independent confirmation. A strong association between soluble P-selectin and certain polymorphisms has been reported, despite no difference in P-selectin between cases with coronary artery disease and controls, and the expected differences in classical risk factors, fibrinogen, ICAM, VCAM and E selectin.\textsuperscript{154} However, this work was performed using serum, with caveats previously mentioned.\textsuperscript{154,155} Preliminary genetics of the PSGL-1 gene indicate considerable polymorphism and an association with the risk of developing cerebrovascular disease.\textsuperscript{155,156}

Platelet microparticles

The sensitivity of flow cytometry permits the identification of small bodies (mean diameter less than 1 \(\mu\)m) that, with the use of antibodies to platelet markers such as glycoprotein Ib and P-selectin, allows their origin to be defined.\textsuperscript{157–160} Compared to healthy controls, increased numbers of microparticles, that have pro-coagulant activity, have been reported in cerebral infarction, uraemia, hypertension,\textsuperscript{46,159} diabetes,\textsuperscript{46,90,160} unstable angina,\textsuperscript{161,162} myocardial infarction\textsuperscript{161} and peripheral artery disease.\textsuperscript{163} Stimuli of microparticle production include ex-vivo aging,\textsuperscript{164} contact with artificial surfaces,\textsuperscript{165} collagen and thrombin,\textsuperscript{166} surgery\textsuperscript{167} and high shear stress.\textsuperscript{168} Although microparticle levels fall with treatment with calcium channel blocker efedidine\textsuperscript{49} or phosphodiesterase inhibitor cilostazol,\textsuperscript{90} their relationship with soluble P-selectin and precise clinical importance is unclear, despite their pro-coagulant nature.\textsuperscript{169}

Inflammation and cytokines

Raised plasma levels of many of the adhesion molecules are taken (mostly by virtue of tissue culture data) to be markers of inflammation. Whilst one of the most widely cited promoters of inflammation, IL-6,\textsuperscript{170} has been quoted as stimulating platelet production,\textsuperscript{171} there is no evidence that it results in increased membrane or soluble P-selectin, and no suggestion that it acts as an acute phase reactant. Although Solheim et al. showed that pravastatin reduced levels of tumour necrosis factor-\(\alpha\), (TNF-\(\alpha\)) it had no effect on soluble P-selectin, C-reactive protein or IL-6.\textsuperscript{172} Schumacher et al.\textsuperscript{173} measured IL-6 and TNF-\(\alpha\) alongside all the soluble adhesion molecules in 193 patients with coronary artery disease and 193 matched controls. As expected, patients had raised levels of all markers and risk factors, but there were no significant multivariate correlations between soluble P-selectin and C-reactive protein, IL-6 or TNF-\(\alpha\). These data, although imperfect (as correlation does not prove causation), fail to support a hypothesis that levels of soluble P-selectin are responsive to inflammatory cytokines. Conversely, Libby and Simon have postulated a contribution by the platelet to inflammatory mechanisms,\textsuperscript{174} by, for example, releasing platelet derived growth factor, although, again, platelet derived growth factor is not generally known for its ability to act as an inflammatory mediator.
What is the practical value of P-selectin for clinicians?

Leaving aside useful lessons regarding the pathophysiology of atherothrombotic disease, how can knowledge of this molecule contribute to improved patient care? The presumption is that high levels of membrane or soluble P-selectin are to be avoided and, if present, minimized. Leaving aside the data from genetics and anticoagulation as currently too preliminary, we identify four possible areas.

1. **As a membrane marker of platelet activation?**

The expression of P-selectin at the surface of the platelet is taken by many workers as a clinical marker of activation, easily detectable with flow cytometry, although it has also been suggested that the model of flow cytometer can influence results. Nevertheless, it may be useful in assessing general platelet activity in, for example, acute thrombotic conditions. Koksch et al. reported a statistically significant association between decreased soluble P-selectin and angiographically-defined peripheral artery disease. Although the authors correctly point out that theirs is the first study to use flow cytometry to verify platelet activity in peripheral atherosclerosis, there are no lack of data implicating this cell in this disease. However, despite these reports, in mice and baboons, platelets continue to circulate and function after they have shed their P-selectin, leading to our concern that not all functional platelets are being defined by this marker.

2. **As a plasma marker of platelet activation?**

Similarly, a large number of workers take ELISA-defined soluble P-selectin to be a plasma marker of platelet activation, and, if so, it may provide useful insights in individual patients or in larger epidemiological studies. However, a significant proportion of our colleagues draw attention to the possibility that some may arise from the endothelium, therefore doubting specificity. Indeed, Sakamaki et al. concluded that their data of an increase in soluble P-selectin in pulmonary hypertension is due to endothelial injury, and that a reduction in raised levels after prostacyclin is due to endothelial injury, concluded that their data of an increase in soluble P-selectin does reflect some aspect of platelet activation, but also as a direct inducer of pro-coagulant activity associated with vascular and thrombotic diseases. If also the case in humans, this may be useful in targeting resources and extra clinical care to high-risk patients. However, are there better markers of disease outcome than soluble P-selectin? For example, raised von Willebrand factor, but not soluble P-selectin, predicted those patients with atrial fibrillation who were at high risk of stroke. In the same cohort, the increase in von Willebrand factor in diabetes (9.6%, P<0.001) was greater than the increase in soluble P-selectin (5.9%, P=0.01) although the reverse was the case in predicting peripheral vascular disease (respectively 6.2% higher versus 14.7% higher).

In the setting of the Emergency Room, it is unclear whether or not soluble P-selectin is a better plasma marker and/or predictor of adverse cardiovascular events than, for example, interleukins, CK-MB or C-reactive protein, or even than classical risk factors such as hypercholesterolaemia or smoking. Hollander et al. enrolling 263 patients, concluded, on the basis of sensitivity and specificity, that neither soluble nor membrane P-selectin had any advantage over the CK-MB in identifying patients with acute coronary syndromes, although Hillis et al. concluded that both soluble P-selectin and troponin I, but not CK-MB, were independent predictors of a serious cardiac event within 3 months of presentation in 126 patients with chest pain of presumed ischaemic origin. Serebruany et al. measured six markers (troponin I, CK, CKMB, myoglobin and soluble and platelet bound P-selectin) in 122 patients presenting with chest pain, of whom 14 were ultimately considered to have AMI, 23 with unstable angina, 16 with heart failure and 69 with non-cardiac chest pain. Of the markers, in Multivariate analysis, myoglobin and platelet P-selectin predicted cardiac origin and AMI, whilst myoglobin and soluble P-selectin predicted heart failure. The diagnostic value of soluble P-selectin in identifying heart failure was substantially increased by considering myoglobin and troponin I measurements. These latter two pilot studies (and some others) certainly provide data warranting a multi-centre trial adequately powered to determine any possible contribution of soluble P-selectin in diagnosis and/or outcome.

3. **As a predictor of adverse outcome?**

Despite the above, whatever its origin, increased levels of soluble P-selectin measured in citrated plasma seem able to predict patients at risk of an adverse cardiovascular event. This may have a direct pathophysiological explanation as mice engineered to have high soluble P-selectin also exhibit a pro-coagulant state, suggesting that this molecule should not only be considered as a marker of platelet activation, but also as a direct inducer of pro-coagulant activity associated with vascular and thrombotic diseases. If also the case in humans, this may be useful in targeting resources and extra clinical care to high-risk patients. However, are there better markers of disease outcome than soluble P-selectin? For example, raised von Willebrand factor, but not soluble P-selectin, predicted those patients with atrial fibrillation who were at high risk of stroke. In the same cohort, the increase in von Willebrand factor in diabetes (9.6%, P<0.001) was greater than the increase in soluble P-selectin (5.9%, P=0.01) although the reverse was the case in predicting peripheral vascular disease (respectively 6.2% higher versus 14.7% higher).

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4. **As a therapeutic target?**

This area is very much in its infancy. Although promising and interesting in vitro and animal data exist suggesting that interference with the P-selectin/PSGL-1 interaction may be beneficial as yet little human work is available. However, a recent ‘human’ experience with
use of a monoclonal antibody to ICAM after ischaemic stroke was, despite promising animal data, disappointing. Similarly, long-term experience with an antibody to ICAM in patients with rheumatoid arthritis has also failed to live up to expectations extrapolated from preliminary and animal data. Nevertheless, inhibition with precisely targeted small peptides or carbohydrate fragments of P-selectin and/or PSGL-1 may be more successful. One of the few recent reports described preliminary data on the pharmacokinetics of a fused rPSGL-immunoglobulin molecule in four non-human species that may provide data for a possible phase 1 trial and thus some therapeutic use.

Summary and conclusions

P-selectin is a component of the membrane of platelet and endothelial intracellular storage organelles, and its appearance on the surface (defined by flow cytometry) is taken to imply activation, under which conditions it mediates cell/platelet adhesion. A soluble form, detectable by ELISA, present in the blood implies increased platelet activation, and raised levels are found in cardiovascular disease and its risk factors. However, although preliminary data suggests these raised levels predict adverse outcome, it is unclear if this provides better prognostic information than other, more established markers. Its role in mediating intercellular adhesion has prompted work on intervening with this process as a new therapy, but human data are not yet available.

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References

nomura s, kanazawa s, fukuhara s. effects of efonidipine on platelet. j clin invest 1998; 102(1):145–52.

hoo y, ley k. adhesion molecules and atherosclerosis. acta physiol scand 2001; 173:35–43.

kriegstein cg, guan y, iizuka y. adhesion molecules and their roles in vascular disease. am j hypertens 2001; 14:445–54.


stenberg pe, mciver rp, shuman ma et al. a platelet alpha granule membrane protein (gmp-140) is expressed on the plasma membrane after activation. j cell biol 1985; 101:880–6.


schmitz g, rothe g, ruf a et al. european working group on clinical cell analysis: consensus protocol for the flow cytometric characterization of platelet function. thromb haemost 1998; 79:885–96.


nomura s, kazazawa s, fujiwara s. effects of efondipine on platelet and monocyte activation markers in hypertensive patients with and without type 2 diabetes mellitus. j hum hypertens 2002; 16:1345–9.


tschoepe d, schluntheiss hp, kolarov p et al. platelet membrane activation markers are predictive for increased risk of acute ischaemic events after ptca. circulation 1993; 88:37–42.


huhe g, abelthauer c, mayer n et al. reduction of platelet activity in type ii hypercholesterolaemia by a hmg-coa reductase inhibitor. thromb res 1999; 95:229–34.


merten m, chow t, hellums jd et al. a new role for p-selectin in shear induced platelet aggregation. circulation 2000; 102:2045–50.


ceci a, pellergrini g, lorentez r et al. p-selectin induces the expression of tissue factor on monocytes. proc natl acad sci usa 1994; 91(19):8767–71.

subramanian m, frenette ps, saffaripour s et al. defects in haemostasis in p-selectin deficient mice. blood 1996; 87:1388–42.


schick pk, konkle ba, he x et al. p-selectin mRNA is expressed at a later phase of megakaryocyte maturation than mRNAs for von willebrand factor and glycoprotein Ib-α. j lab clin med 1993; 121(5)(5):71–24.

ishiwata n, takio k, katayama m et al. alternatively spliced isoform of p-selectin in present in vivo and is a soluble molecule. j biol chem 1994; 269:23708–15.


kosteljik eh, fijnheer r, niewehuis hk et al. soluble p-selectin as a parameter for platelet activation during storage. thromb haemost 1996; 76:1086–9.

fijnheer r, fijnheer cmj, korteweg j et al. the origin of p-selectin as a circulating plasma protein. thromb haemost 1997; 77:1081–5.


mciver rp, backstead jh, moore kl et al. gmp-140, a platelet alpha granule membrane proteins, is also synthesized by vascular endothelial cells and is localized in weibel-palade bodies. j clin invest 1989; 84:92–9.


chong bh, murray b, berndt mc et al. plasma p-selectin is increased in thrombotic consumptive platelet disorders. blood 1994; 83(6):1535–41.

katayama m, handa m, araki y et al. soluble p-selectin is present in normal circulation and its plasma level is elevated in patients with thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome. br j haematol 1993; 84(4):702–10.

banu n, avraham s, avraham hk. p-selectin, and not e-selectin, negatively regulates murine megakaryocytepoiesis. j immunol 2002; 169(8):4579–85.

jilma b, eichler hg, becherer a et al. kinetics of circulating selectin levels during bone marrow aplasia. eur j haematol 1998; 61(1):36–41.


kaikita k, ogawa h, yase h et al. soluble p-selectin is released into the coronary circulation after coronary spasm. circulation 1995; 92:1726–30.


frijns cmj, kappelle lj, van ginj j et al. soluble adhesion molecules reflect endothelial cell activation in ischaemic stroke and in carotid atherosclerosis. stroke 1997; 28:2214–8.


