



Role of von Willebrand factor in tumour cell-induced platelet aggregation: differential regulation by NO and prostacyclin

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1 We have studied the effects of a novel agonist, solid-phase von Willebrand Factor (sVWF), on tumour cell-induced platelet aggregation (TCIPA).

2 Washed platelet suspensions were obtained from human blood and the effects of HT-1080 human fibrosarcoma cells and sVWF on platelets were studied using aggregometry, phase-contrast microscopy, and flow cytometry.

3 Incubation of platelets with sVWF ($1.2 \mu\text{g ml}^{-1}$) and HT-1080 cells ($5 \times 10^3 \text{ ml}^{-1}$) resulted in a two-phased reaction characterized first by the adhesion of platelets to sVWF, then by aggregation.

4 TCIPA in the presence of sVWF was inhibited by S-nitroso-glutathione (GSNO, $100 \mu\text{M}$) and prostacyclin (PGI_2 , 30 nM).

5 Platelet activation in the presence of tumour cells and sVWF resulted in the decreased surface expression of platelet glycoprotein (GP)Ib and up-regulation of GPIIb/IIIa receptors.

6 Pre-incubation of platelets with PGI_2 (30 nM) resulted in inhibition of sVWF-tumour cell-stimulated platelet surface expression of GPIIb/IIIa as measured by flow cytometry using antibodies directed against both non-activated and activated receptor. In contrast, GSNO ($100 \mu\text{M}$) did not affect sVWF-tumour cell-stimulated platelet surface expression of GPIIb/IIIa.

7 Flow cytometry performed with PAC-1 antibodies that bind only to the activated GPIIb/IIIa revealed that GSNO ($100 \mu\text{M}$) caused inhibition of activation of GPIIb/IIIa.

8 The inhibitors exerted no significant effects on TCIPA-mediated changes in GPIb.

9 Thus, sVWF potentiates the platelet-aggregatory activity of HT-1080 cells and these effects appear to be mediated *via* up-regulation of platelet GPIIb/IIIa.

10 Prostacyclin and NO inhibit TCIPA-sVWF-mediated platelet aggregation. The mechanisms of inhibition of this aggregation by PGI_2 differ from those of NO.

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Abbreviations: GPIb, Glycoprotein Ib; GPIIb/IIIa, glycoprotein IIb/IIIa; GSNO, S-nitroso-glutathione; PGI_2 , prostacyclin; sVWF, solid-phase von Willebrand factor; TCIPA, tumour cell-induced platelet aggregation

Introduction

During the metastatic cascade the haematogenous spread of cancer cells can be accomplished by tumour cell-containing thrombi. Cancer cells have the ability to aggregate platelets (Gasic *et al.*, 1968; 1973), which are the major components of arterial thrombi, and this action correlates with the metastatic potential of tumour cells (Radomski *et al.*, 1991). Tumour cell-induced platelet aggregation (TCIPA) confers a number of advantages to the survival of cancer cells in the vasculature and to metastasis. Once covered with a coat of platelets the tumour cell acquires the ability to elude the body's defence system (Shau *et al.*, 1993; Philippe *et al.*, 1993). In addition, the platelets may shield the cancer cells from the high shear forces seen in flowing blood that could potentially damage the tumour cell. Moreover, aggregated

platelets release a number of growth factors that can be used by tumour cells for growth (Honn *et al.*, 1992). Furthermore, tumour cell–platelet aggregates embolize in the microvasculature, allowing tumour cells to adhere to damaged endothelium at an extravasation site (Mehta, 1984; Crissman *et al.*, 1985). The pathways and mechanisms that mediate TCIPA have now been studied and are likely to involve the release of thromboxane A_2 , ADP, and matrix metalloproteinase-2 from the aggregates (Bastida *et al.*, 1982; Pearlstein *et al.*, 1981; Honn *et al.*, 1987; Steinert *et al.*, 1993; Jurasz *et al.*, 2001). However, little is known about the interactions of platelet–tumour cell aggregates with von Willebrand factor (VWF) and its platelet receptors glycoprotein (GP)Ib and GPIIb/IIIa (Weiss *et al.*, 1974; Nurden & Caen, 1974; Caen & Rosa, 1995; Ruggeri, 1999). Von Willebrand factor is a major adhesion protein that mediates platelet attachment to the vascular wall and this protein can interact both with GPIb and GPIIb/IIIa receptors. VWF is a large multimeric

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protein found in plasma, platelets, endothelial cells, and in the subendothelium.

GPIIb/IIIa is a platelet specific surface transmembrane receptor that belongs to the integrin superfamily. It is the most abundant platelet receptor with roughly 80,000 copies on the surface of each platelet. In addition, platelets contain a pool of GPIIb/IIIa stored in platelet α granules (Wagner *et al.*, 1996) that is released onto the platelet surface upon platelet activation. Furthermore, GPIIb/IIIa undergoes activation and a conformational change that allows binding to its primary ligands fibrinogen and VWF (Ruggeri *et al.*, 1999) to mediate platelet-platelet and platelet-vessel wall interactions.

GPIb belongs to the leucine-rich superfamily of receptors. It complexes with glycoproteins IX and V and forms an active receptor. The major ligand of GPIb is VWF and its binding to the receptor mediates platelet adhesion and arrest at the damaged fragment of the vessel wall. However, shear forces generated under conditions of flowing blood may also result in VWF-mediated platelet aggregation (Ruggeri, 1999).

Agonist-induced platelet surface receptor expression can be modulated by a number of anti-platelet agents. Nitric oxide, a well-known inhibitor of platelet activation and aggregation (Radomski *et al.*, 1987a,b; 1991), has been shown to affect GPIIb/IIIa receptor expression during platelet activation (Salas *et al.*, 1994). Moreover, prostacyclin (PGI₂), the most potent known inhibitor of platelet aggregation (Moncada *et al.*, 1976), prevents the mobilization of platelet GPIIb/IIIa in thrombin-activated platelets (Graber & Hawiger, 1982). In addition, it has been shown that GPIb/IX/V can be modified by the phosphorylation of Ser166 by cyclic AMP-dependent kinase (Wyler *et al.*, 1986; Wardell *et al.*, 1989) that is activated by PGI₂.

Recently, Stewart *et al.* (1997) reported that VWF when immobilized on polystyrene beads acts as a novel solid-phase agonist (sVWF) in human platelet-rich plasma. In the paper accompanying this work we have found that sVWF stimulates both adhesion and agonist-induced aggregation of human platelets and that these effects could be regulated by NO and PGI₂ (Radomski *et al.*, in preparation). Therefore, the aim of our present study was to investigate the effects of sVWF, on TCIPA. In addition, we studied the expression of platelet GPIIb/IIIa and GPIb during these reactions and the regulation of these two receptors by NO and PGI₂.

Methods

Blood platelets

Blood was collected from healthy volunteers who had not taken any drugs for 14 days prior to the study. Washed platelet suspensions ($2.5 \times 10^{11} \text{ l}^{-1}$) were prepared as previously described (Radomski & Moncada, 1983).

Tumour cell culture

A human tumour cell line HT-1080 fibrosarcoma was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) and prepared as previously described

(Jurasz *et al.*, 2001). The cells were resuspended in Tyrode's solution at a concentration of $5 \times 10^5 \text{ ml}^{-1}$ (Jurasz *et al.*, 2001). All cell culture reagents were purchased from Sigma (Oakville, ONT, Canada).

Platelet aggregation

Washed platelets were pre-incubated for 2 min at 37°C in a Chronolog whole blood ionised calcium lumi-aggregometer. Platelet aggregation was then initiated by the addition of HT-1080 cells ($5 \times 10^3 \text{ cells ml}^{-1}$) and monitored by Aggro-Link software (Jurasz *et al.*, 2001). Platelet aggregation was measured as an extent of light transmittance and then expressed as a per cent of maximal stimulus taken at a time point when the maximal stimulus reached 50% transmittance (Jurasz *et al.*, 2001). Platelet samples for flow cytometry were taken at this time point.

Flow cytometry

Platelet flow cytometry was performed using a Becton Dickinson flow cytometer (FACSCalibur) equipped with a 488 nm wavelength argon laser, 525 and 575 nm band pass filters for the detection of fluorescein isothiocyanate and R-phycoerythrin fluorescence, and with Cell Quest software. Flow cytometry was performed on both single and double stained platelet samples. To account for spectral overlap between fluorescein isothiocyanate and R-phycoerythrin labels software compensation was performed in experiments using double stained platelet samples. To minimize the presence of aggregates in samples of platelets (10 μl of suspension) and fluorescent-labelled antibodies (10 μl containing 1 μg of anti-GPIIb and GPIb antibodies, and 10 μl containing 0.25 μg of PAC-1 antibodies) were diluted 10 fold using physiological saline. Platelets were identified by forward and side scatter signals. Ten to twenty thousand platelet specific events were initially analysed by the cytometer. Non-activated and activated platelets were gated so as not to analyse platelet aggregates and microparticles. Furthermore, the gated whole platelet population was arbitrarily sub-gated into large-, medium-, and small-sized platelet populations (Radomski *et al.*, in preparation). The gates were then analysed for mean fluorescence.

Microscopy

Tumour cell-platelet samples were viewed using phase-contrast microscopy equipped with a Nikon camera (Jurasz *et al.*, 2001).

Reagents

Prostacyclin and S-nitroso-glutathione (GSNO) were obtained from Sigma (Oakville, ONT, Canada). These reagents were incubated with platelets for 2 min prior to the addition of tumour cells. Fluorescein isothiocyanate conjugated (FITC) monoclonal mouse anti-platelet GPIIb (human CD41) antibodies and R-phycoerythrin conjugated (RPE) monoclonal mouse anti-platelet GPIb (human CD42b) antibodies were obtained from DAKO (Mississauga, ONT, Canada). PAC-1 FITC monoclonal mouse anti-platelet

GPIIb/IIIa antibodies were obtained from Becton Dickinson (San José, CA, U.S.A.). Von Willebrand factor coated polystyrene beads (Stewart *et al.*, 1997) were provided by Thrombotics Inc (Edmonton, AB, Canada). The beads were washed and resuspended in equivalent volume of saline and used for the experiments.

Statistics

Statistics were performed using Graph Pad Software Prism 3.0 (San Diego, CA, U.S.A.). All means are reported with standard error. One-way analysis of variance (ANOVA), Tukey–Kramer multiple comparisons test, and paired

Student *t*-tests were performed where appropriate, and a *P*-value of less than 0.05 was considered as significant.

Results

TCIPA in the presence of sVWF

Incubation of HT-1080 cells with platelets resulted in TCIPA (Figure 1). This effect was potentiated in the presence of sVWF (Figure 1), as evidenced by the faster onset of aggregation. Furthermore, TCIPA in the presence of sVWF was two-phased. The first phase marked platelet adhesion to

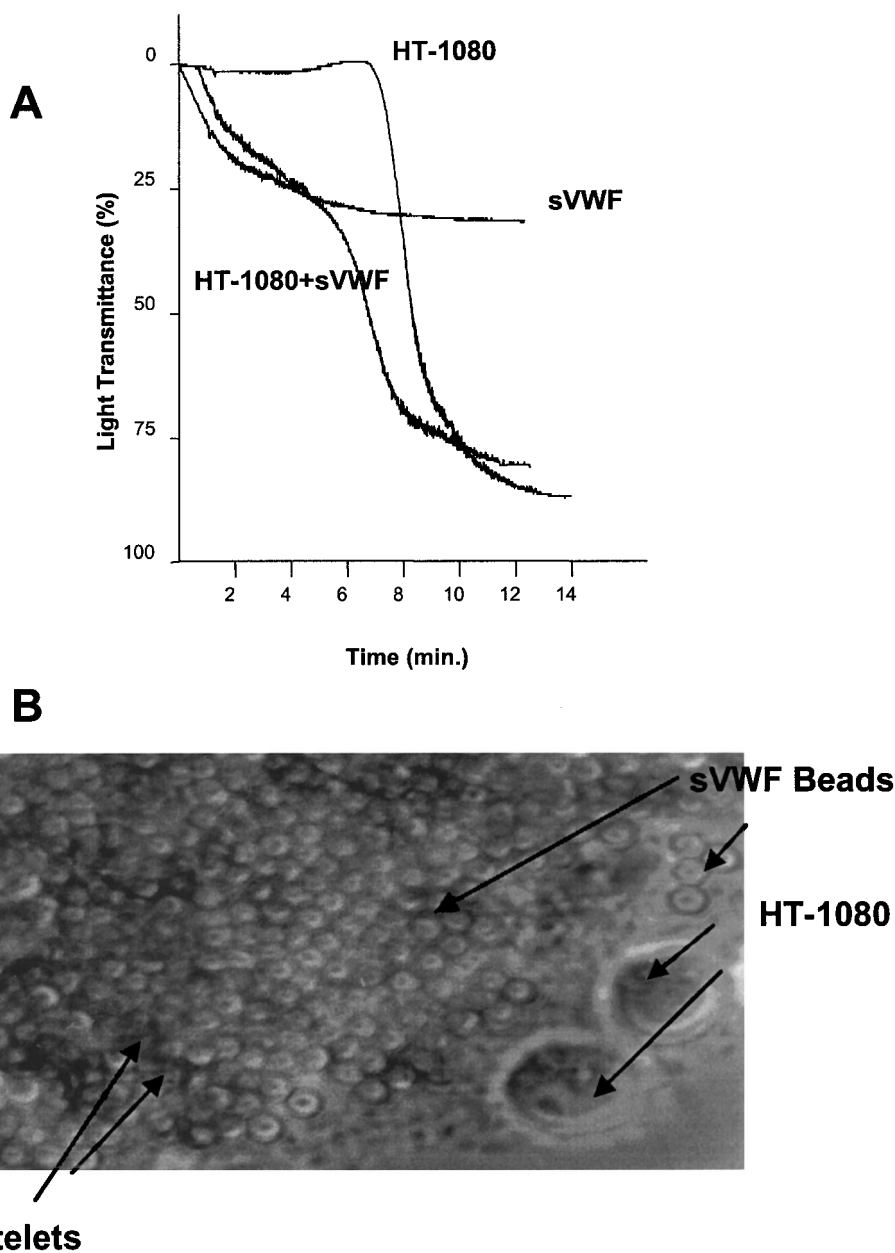


Figure 1 (A) Representative traces showing TCIPA in the presence or absence of sVWF ($1.6 \mu\text{g ml}^{-1}$) (representative of six experiments). TCIPA was induced by HT-1080 cells ($5 \times 10^3 \text{ cells ml}^{-1}$). (B) Phase-contrast microscopy of HT-1080 induced TCIPA in the presence of sVWF, $\times 400$.

the immobilized sVWF and the second phase platelet–tumour cell aggregation (Figure 1A,B).

Effects of GSNO and PGI₂

GSNO (100 μM) and PGI₂ (30 nM) both significantly ($P < 0.01$ and $P < 0.001$, respectively; $n = 6$) inhibited HT-1080 induced platelet aggregation (Figure 2A,B). Preincubation of platelets with GSNO (100 μM) and PGI₂ (30 nM) also significantly ($P = 0.0016$; $n = 6$, and $P = 0.0003$; $n = 3$) inhibited TCIPA in the absence of sVWF. The latter observation is consistent with our previous findings (Jurasz *et al.*, 2001).

GPIb and GPIIb/IIIa during the adhesion phase of TCIPA

In the absence of HT-1080 cells, sVWF caused an increase in the surface expression of platelet GPIb, as evidenced by a significant ($P = 0.0283$; $n = 4$) increase in mean fluorescence (Figure 3A). However, during the platelet adhesion phase of TCIPA in the presence of sVWF, HT-1080 cells caused a decrease in the platelet surface expression of GPIb, as compared to sVWF alone. This was evidenced by the significant ($P = 0.0231$, $n = 4$) decrease in mean fluorescence (Figure 3B).

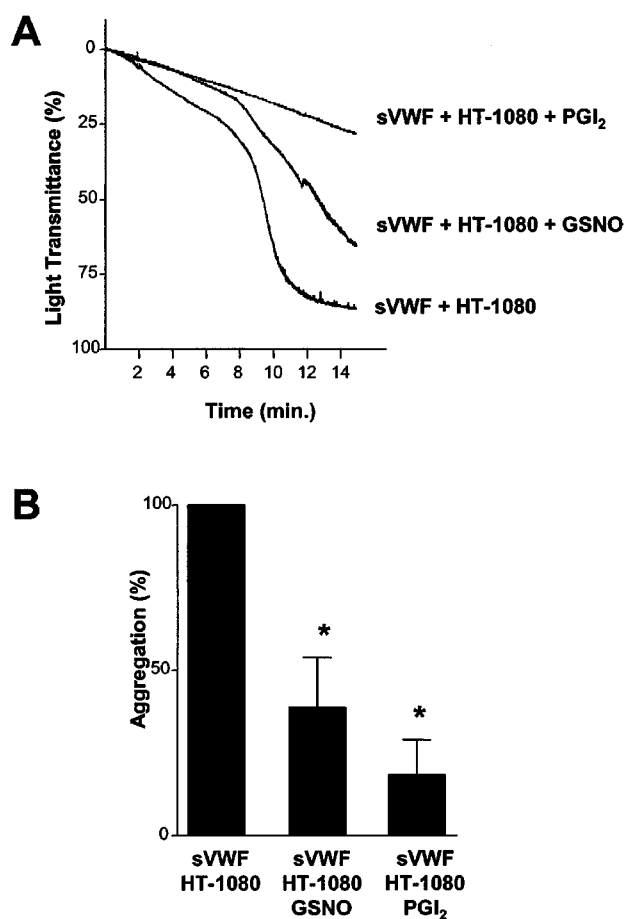


Figure 2 Representative traces (A) and the statistical analysis (B) showing the effects of GSNO (100 μM) and PGI₂ (30 nM) on TCIPA in the presence of sVWF (1.6 $\mu\text{g ml}^{-1}$). TCIPA was induced by HT-1080 cells (5×10^3 cells ml^{-1}). Bars are mean \pm s.e.mean from six separate experiments. * $P < 0.05$, treatments versus control.

No significant changes ($P > 0.05$, $n = 3$) in the surface expression of GPIIb/IIIa were detected during these experiments.

GPIIb/IIIa and GPIb during the aggregation phase of TCIPA

During the aggregation phase of TCIPA in the presence of sVWF GPIIb/IIIa expression significantly ($P < 0.01$, $n = 4$) increased (Figure 4A). Similar, during TCIPA in the absence of sVWF there was a significant ($P = 0.0010$, $n = 9$) increase in the platelet surface expression of GPIIb/IIIa. Platelet activation with sVWF in the absence of HT-1080 cells did not cause any changes in GPIIb/IIIa (Figure 4A).

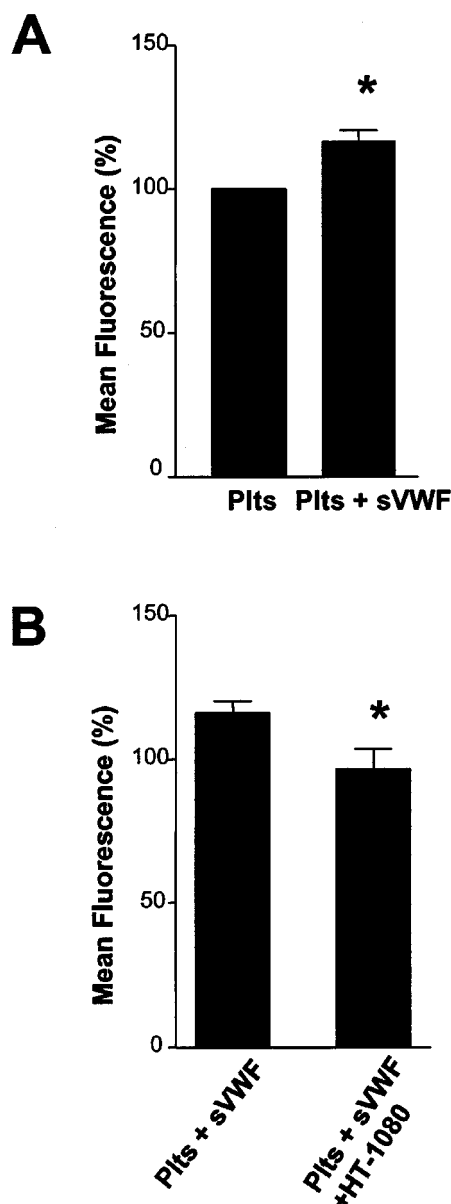


Figure 3 Effects of sVWF (1.6 $\mu\text{g ml}^{-1}$) on platelet surface expression of GPIb in the absence (A) or presence (B) of HT-1080 cells. TCIPA was induced by HT-1080 cells (5×10^3 cells ml^{-1}). Bars are mean \pm s.e.mean from four separate experiments. * $P < 0.05$, treatment versus control.

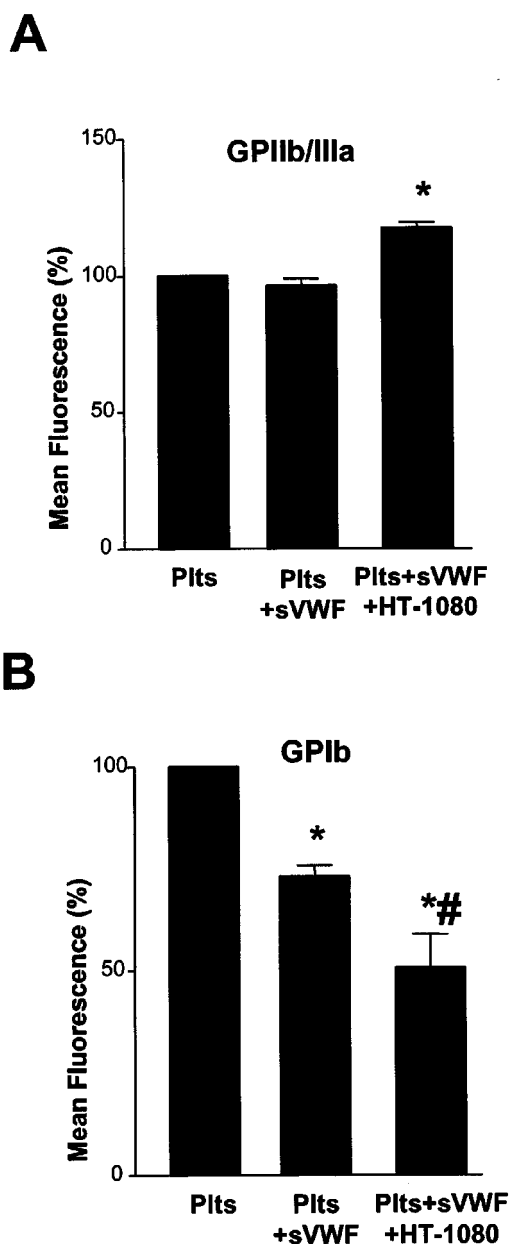


Figure 4 Effects of HT-1080 cells and sVWF ($1.6 \mu\text{g ml}^{-1}$) on GPIIb/IIIa and GPIb expression during the aggregation phase of TCIPA ($5 \times 10^3 \text{ cells ml}^{-1}$). Bars are mean \pm s.e. mean from four separate experiments. * $P < 0.05$, treatments versus control. # $P < 0.05$, platelets and VWF versus platelets and VWF and HT-1080 cells.

TCIPA in the presence of sVWF resulted in a significant ($P < 0.001$, $n = 4$) decrease in platelet surface GPIb (Figure 4B). TCIPA in the absence of sVWF also led to a significant ($P = 0.0001$, $n = 9$) decrease in platelet surface expression of GPIb (Figure 4B).

Effects of GSNO and PGI₂ on both non-activated and activated GPIIb/IIIa

Flow cytometry performed on platelets pre-incubated with GSNO ($100 \mu\text{M}$) or PGI₂ (30 nM) and then activated by HT-1080 cells and sVWF was analysed by examining different

sized platelet populations. PGI₂, but not GSNO, in large, medium and small platelet subpopulations significantly ($P < 0.01$, $P < 0.05$ and $P < 0.01$, respectively, $n = 6$) inhibited HT-1080-mediated increase in the surface number of platelet GPIIb/IIIa (Figure 5A–C). In contrast, GSNO failed to prevent the increase in platelet surface GPIIb/IIIa ($P > 0.05$; $n = 6$) (Figure 5A–C).

Effects of GSNO on activated GPIIb/IIIa

Flow cytometry performed with the PAC-1 antibody that recognizes only the activated GPIIb/IIIa showed that in large, medium, and small platelet subpopulations GSNO ($100 \mu\text{M}$) significantly ($P = 0.0103$, $P = 0.0176$ and $P = 0.0110$, respectively, $n = 3$) inhibited the activation of GPIIb/IIIa during TCIPA (Figure 6A–C). GSNO ($100 \mu\text{M}$) caused also inhibition of activation of GPIIb/IIIa during TCIPA in the absence of VWF, as evidenced by the significant ($P = 0.0284$, $n = 6$) decrease in mean fluorescence.

Discussion

The objective of our investigation was to study TCIPA in the presence of sVWF, a novel solid-phase agonist (Stewart *et al.*, 1997). As VWF is a major adhesion protein contributing to the interactions between platelets and the vascular wall (Ruggeri *et al.*, 1999), the use of sVWF added yet another dimension to studies on TCIPA that have considered until now only the platelet–tumour cell relationship (Honn *et al.*, 1981; Radomski *et al.*, 1991; Jurasz *et al.*, 2001). In addition, we investigated the effects of NO and PGI₂, two known inhibitors of platelet aggregation (Radomski *et al.*, 1987a,b; Moncada *et al.*, 1976) and TCIPA (Radomski *et al.*, 1991; Jurasz *et al.*, 2001; Honn *et al.*, 1981; Menter *et al.*, 1984) on the TCIPA-sVWF-induced platelet activation.

As reported before, (Jurasz *et al.*, 2001), human fibrosarcoma cells HT-1080 caused TCIPA. Preincubation of platelet samples with sVWF resulted in potentiation of TCIPA. This reaction consisted of two phases: the first dependent upon platelet adhesion to sVWF, and the second characterized by formation of tight sVWF–platelet–tumour cell aggregates. These data show that VWF appears to play a crucial role in the interactions between cancer cells and platelets *in vitro*, and most likely *in vivo*. In this context, it is interesting to note that numerous studies have shown that cancer patients have elevated levels of plasma VWF, in addition to other coagulation factors, which all may contribute to thrombotic risk. These include laryngeal (Paczuski *et al.*, 1999), renal (Oleksowicz *et al.*, 1999), colorectal (van Duijnhoven *et al.*, 1993), cervical (Gadducci *et al.*, 1993), prostate (Ablin *et al.*, 1988), and head and neck cancers (Sweeney *et al.*, 1990). Moreover, Oleksowicz *et al.* (1999) have shown that patients with metastatic disease have elevated levels of highly polymeric forms of VWF due to a deficiency of VWF-processing protease. Furthermore, increased thrombotic readiness detected in cancer patients may be due to the elevated plasma levels of VWF (Green *et al.*, 1997). In addition, patients with malignant breast cancer have increased VWF content in the cytosol of the malignant tissue (Pratt *et al.*, 1989). Importantly, it has been demonstrated that the staging and tumour size correlate with disease

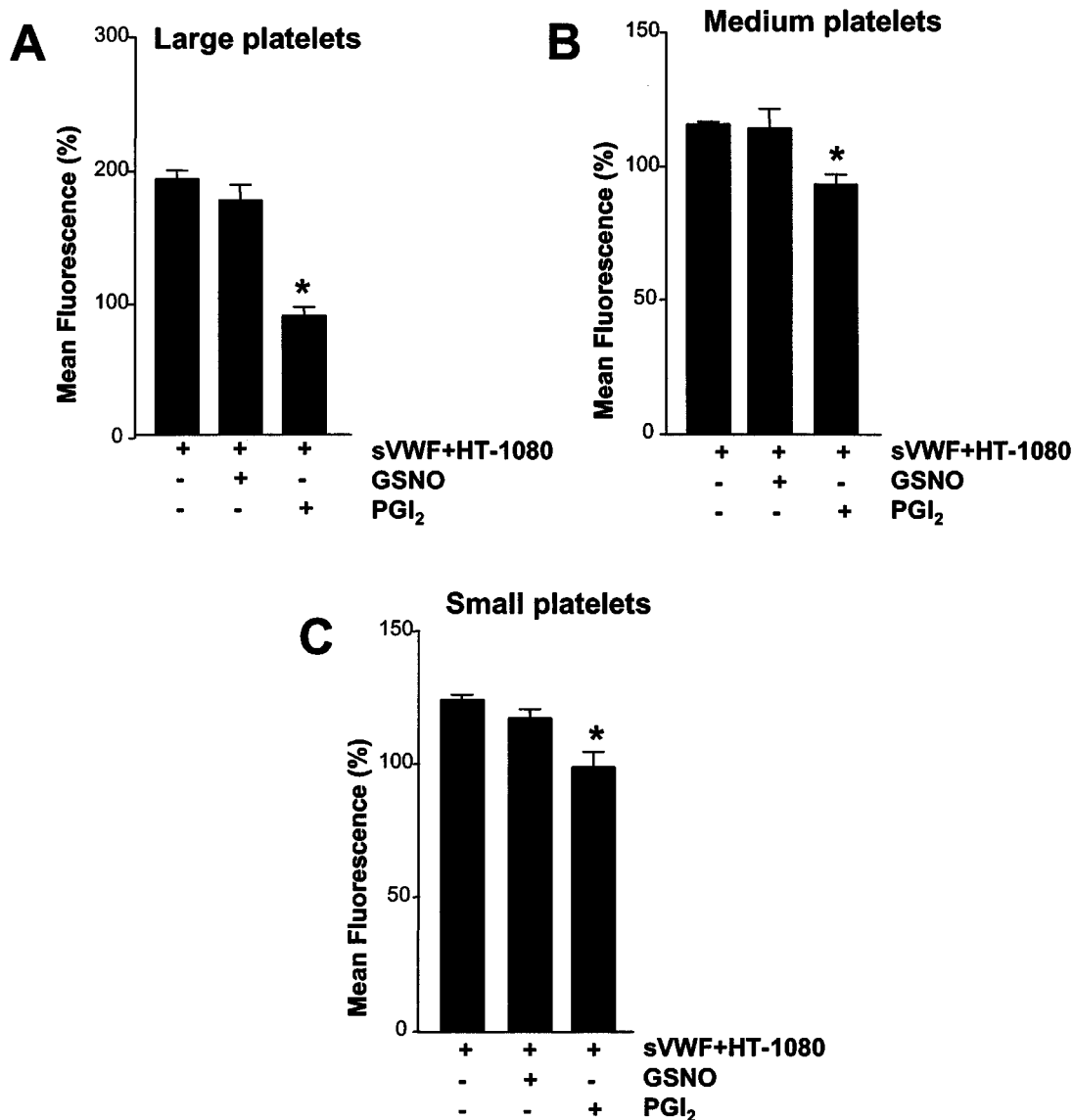


Figure 5 Effects of GSNO (100 μ M) and PGI₂ (30 nM) on expression of platelet GPIIb/IIIa in different-sized platelet populations during TCIPA. (A) Large platelets, (B) Medium platelets, and (C) Small platelets. TCIPA was induced by HT-1080 cells (5×10^3 cells ml^{-1}) and sVWF (1.6 $\mu\text{g ml}^{-1}$). Bars are mean \pm s.e.mean from six separate experiments. * $P < 0.05$, treatments versus control. + and - denote the presence or absence of treatments, respectively.

progression whereby patients with higher plasma VWF have more advanced disease (Paczuski *et al.*, 1999; Gadducci *et al.*, 1993; Pratt *et al.*, 1989). Experimentally, co-culture of human HRT-18 colon cancer cells with human umbilical vein endothelial cells leads to an increase in VWF release from the endothelial cells and this may lead to enhanced platelet adhesion (Morganti *et al.*, 1996). Finally, platelet aggregation induced by human MCF breast cancer cells is increased in cancer patients with elevated levels of VWF (Oleksowicz *et al.*, 1999). The mechanisms that lead to an increase in plasma VWF in cancer patients remain unclear; however, they may include damage to the endothelium, platelet activation and aggregation or tumour angiogenesis. Whatever the mechanisms underlie increased release of VWF, clinical and experimental evidence strongly indicate that this protein, along with others such as tissue factor, plays an integral role in haematogenous spread of cancer.

We have also studied the mechanisms of the activator effects of sVWF on TCIPA focusing upon two major platelet receptors GPIb and GPIIb/IIIa.

In agreement with our previous work (Radomski *et al.*, in preparation), preincubation of platelets with sVWF alone caused increased surface expression of GPIb. This enhanced expression of GPIb is likely to support platelet adhesion to this ligand (Radomski *et al.*, in preparation) in light of GPIb α /VWF-mediated tethering (Ruggeri, 1999). In contrast, TCIPA resulted in a significant reduction of GPIb expression on platelet surface both in the presence or absence of sVWF. Decreased surface expression of GPIb has been already reported as a result of thrombin-induced platelet aggregation or the exposure of platelet to the foreign surface (Keh *et al.*, 1996; Michelson *et al.*, 1996; Mellgren *et al.*, 1995). This reduction could be a result of receptor internalization or proteolysis and shedding (Mellgren *et al.*, 1995, Hughes *et al.*,

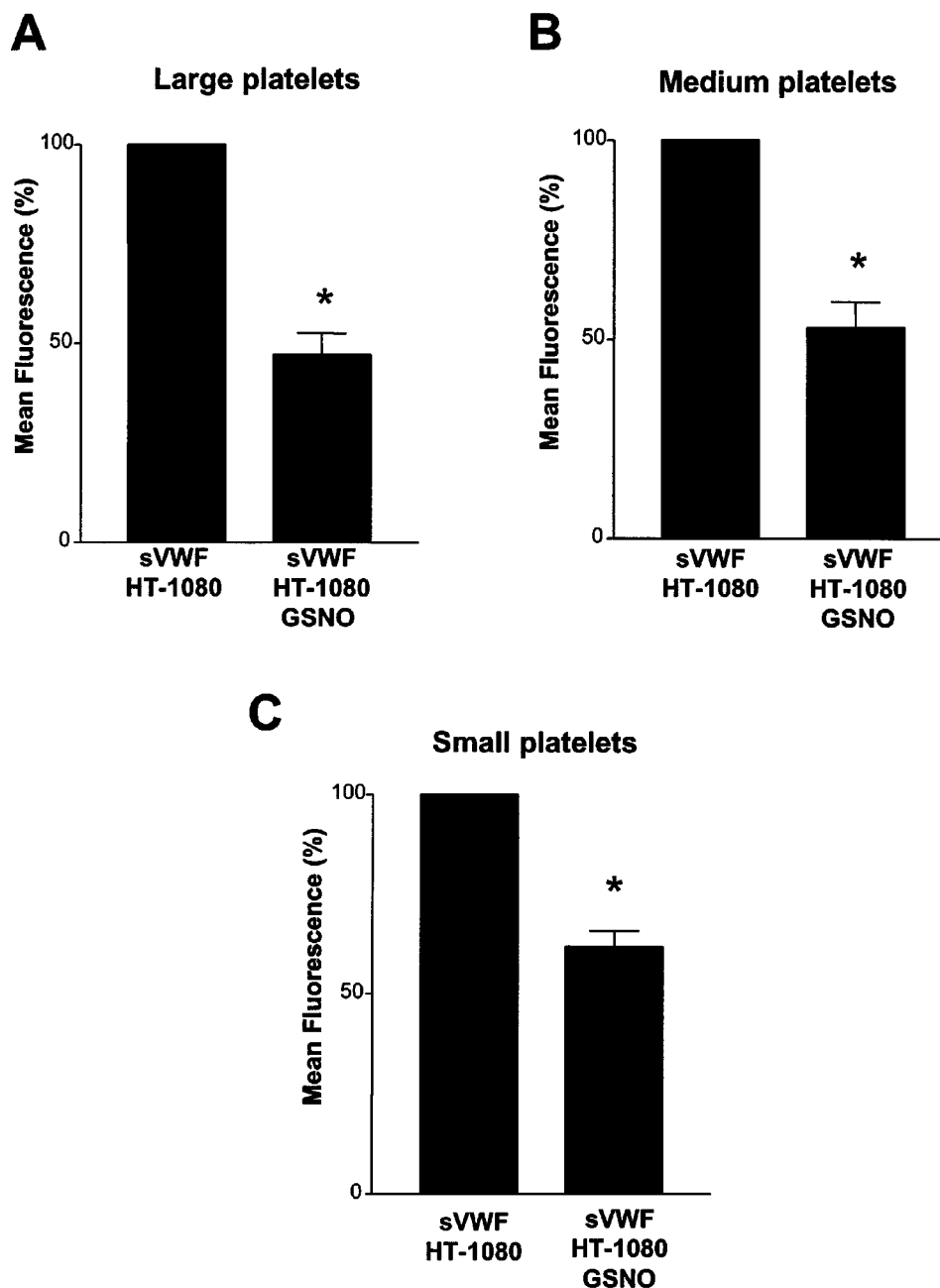


Figure 6 Effects of GSNO (100 μ M) on the activation of platelet GPIIb/IIIa during TCIPA in the presence of sVWF measured with the PAC-1 antibody. TCIPA was induced by HT-1080 cells (5×10^3 cells ml^{-1}) and sVWF (1.6 $\mu\text{g ml}^{-1}$). Bars are mean \pm s.e.mean from three separate experiments. * $P < 0.05$, treatment versus control.

2000; Bergmeier *et al.*, 2000; Kinlough-Rathbone *et al.*, 2000). The biological significance of reduced expression of GPIb during aggregatory reactions is unclear.

In contrast to GPIb, TCIPA-sVWF-mediated platelet activation was clearly associated with increased surface expression of GPIIb/IIIa emphasizing, once again, an important role of this receptor in platelet activation by tumour cells (Jurasz *et al.*, 2001).

Platelet activation induced by tumour cells in the presence of sVWF was inhibited by NO and prostacyclin highlighting further the regulatory role of these mediators in the interactions between platelets, the vessel wall and tumour

cells. Others and we have previously shown that these inhibitors down-regulate TCIPA (Honn *et al.*, 1981; Radomski *et al.*, 1991; Jurasz *et al.*, 2001). Since the regulatory effects of NO and PGI_2 on agonist-induced platelet aggregation are dependent on modulation of platelet receptor glycoprotein function, we investigated the effects of these inhibitors on TCIPA-sVWF-stimulated changes in GPIb and GPIIb/IIIa expression. PGI_2 inhibited the surface expression of GPIIb/IIIa, while GSNO did not. Since both inhibitors reduced TCIPA-sVWF-stimulated platelet activation, differential effects of NO and PGI_2 on GPIIb/IIIa expression were surprising. Therefore, we investigated

whether GSNO had any effects on the activation of GPIIb/IIIa using the PAC-1 antibody, which recognizes the activated form of GPIIb/IIIa. The study revealed that NO inhibited the activation of GPIIb/IIIa caused by cancer cells. Recently, Keh *et al.* (1996) have shown that GSNO inhibits both the increased surface expression and activation of GPIIb/IIIa in platelets stimulated by thrombin. Our own data show that GSNO only inhibited GPIIb/IIIa activation. This discrepancy may be due to procedures used for sample preparation and/or the nature of the platelet activating agents. To measure total GPIIb/IIIa by flow cytometry Keh *et al.* (1996) fixed their platelet samples and stored them at 4°C for up to 4 h. Measurement of activated GPIIb/IIIa with the PAC-1 antibody, in our experiments, was performed on platelet samples that were not fixed. Fixation of platelets may alter platelet surface receptor expression and/or impair fluorescence intensity. The timing of flow cytometry assays of non-fixed samples is crucial; therefore, all our measurements were performed precisely at 5 min after sample acquisition. Furthermore, the discrepancy in results may arise from the differences between platelet activation by thrombin versus HT-1080 fibrosarcoma cells. Indeed, we have shown that platelet activation by HT-1080 cells is a complex process that involves the activation of the ADP, thromboxane, and MMP-2 pathways of platelet aggregation (Jurasz *et al.*, 2001). Activation and aggregation of platelets by cancer cells is not only a complex process, but also a very potent one. Indeed, 10³ cancer cells could induce aggregation of 10⁸ platelets. Thus, NO may not be able to inhibit up-regulation of platelet surface GPIIb/IIIa induced by HT-1080 cells, but it does inhibit the conformational change in GP IIB/IIIa that precedes platelet aggregation. In contrast to NO, PGI₂ down-regulated an increase in GPIIb/IIIa surface expression induced by TCIPA-sVWF.

Interestingly, it has been shown that tyrosine phosphorylation of platelet proteins associated with aggregation is inhibited by cyclic AMP-, but not cyclic GMP-elevating agents (Pumiglia *et al.*, 1990). As tyrosine phosphorylation is linked to GPIIb/IIIa function (Golden *et al.*, 1990; Parise *et al.*, 1990), a differential regulation of function of this receptor by NO and PGI₂ may be explained by the differences in

signal transduction mechanisms operated by the PGI₂-cyclic AMP and NO-cyclic GMP systems. Neither PGI₂ nor NO exerted any significant effects on reduction of GPIb surface expression caused by TCIPA-sVWF. This data indicates that inhibition of TCIPA-sVWF by NO and PGI₂ is related to their interactions with GPIIb/IIIa rather than GPIb.

Platelet activation leads to the formation of platelet particles of various sizes. Therefore, in our flow cytometry assay we have analysed the effects of stimulators of aggregation (HT-1080 cells and sVWF) and inhibitors (PGI₂ and GSNO) on large, medium, and small sized platelet populations. We found that these activators and inhibitors affected different sized platelet populations in the same manner. We feel that our model of TCIPA including sVWF may be of use to understand further the process of haematogenous metastasis of cancer. It is likely that a haematogenous metastasizing cancer cell should follow the path of least resistance (Cotmore & Carter, 1973). This means that the cell will arrest and extravasate at a site in the vasculature where vascular integrity has been disrupted. This site of vascular injury will undoubtedly already have platelets adhering and aggregating to it in an effort to control bleeding and to repair the damaged vessel. Therefore, our data suggests that the ability of the tumour cell to aggregate platelets and consequently upregulate platelet surface GPIIb/IIIa gives the tumour cell-platelet aggregate the advantage of engaging platelets already in the process of aggregation at the site of vascular injury.

In conclusion, we have demonstrated that sVWF amplifies TCIPA. The effects of sVWF are largely mediated *via* increased expression of GPIIb/IIIa in platelets. Finally, these actions of sVWF are modulated by NO and prostacyclin.

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