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ORIGINAL ARTICLE

The effects of GPIIb-IIIa antagonists and a combination of three other antiplatelet agents on platelet–leukocyte interactions

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SUMMARY

The effects of the GPIIb-IIIa antagonists abciximab and MK-852 on platelet–leukocyte interactions *in vitro* were studied and the results compared with those obtained with a combination of aspirin, dipyridamole and AR-C69931 (Asp/Dip/AR-C). Platelet–monocyte (P/M) and platelet–neutrophil (P/N) conjugate formation increased when blood was stirred or a platelet agonist was added. Leukocyte activation also occurred as judged by expression of surface tissue factor antigen and CD11b. Abciximab and MK-852 potentiated P/M, especially when collagen was used. They also increased the amount of tissue factor on the monocytes, but not CD11b. The Asp/Dip/AR-C did not enhance P/M or tissue factor exposure. Augmented tissue factor expression on monocytes in the presence of a GPIIb-IIIa antagonist may be relevant to the increased mortality associated with trials of such antagonists when given orally in patients with vascular disease. The Asp/Dip/AR-C was superior to abciximab and MK-852 in inhibiting platelet and leukocyte function.

Introduction

Both platelet aggregation and fibrin formation contribute to the thrombotic occlusions that give rise to acute manifestations of arterial vascular disease[1–4]. Cell and tissue damage leads to release of platelet activators such as collagen, adenosine diphosphate (ADP) and thromboxane (TxA_2), which induce platelet aggregation, and release of tissue factor results in thrombin generation which causes further platelet aggregation and fibrin formation. Antiplatelet agents such as aspirin, clopidogrel and dipyridamole reduce the incidence of acute thrombosis[3,5]. Such agents inhibit platelet activation, and the platelet aggregation that follows, by blocking the effects of one or more platelet activating agents. Aspirin blocks synthesis of TxA_2. Clopidogrel is an ADP receptor antagonist. Dipyridamole affects platelet activation indirectly through a mechanism involving intracellular cyclic 3′,5′-adenosine monophosphate. Such agents reduce the availability of activated GPIIb-IIIa on the platelet surface, subsequent binding of fibrinogen and the platelet aggregation that results. There is clinical evidence that combining antiplatelet agents with different modes of action is

more antithrombotic than using individual agents alone[6–8].

Platelet aggregation can also be inhibited via direct GPIIb-IIIa blockade using agents such as abciximab, eptifibatide and tirofiban[9]. These do not prevent the platelet activation that occurs when platelets are stimulated by an agonist, but they prevent fibrinogen binding to activated GPIIb-IIIa[9]. GPIIb-IIIa receptor antagonists used intravenously in the setting of percutaneous coronary interventions and acute ischaemic syndromes produce risk reductions of up to 50%[10,11]. However, GPIIb-IIIa receptor antagonists that have been administered orally (xemilofiban, orbofiban and sibrafiban) have not been shown to produce clear treatment benefit[12]. Indeed, an increased mortality has been observed in those patients receiving oral GPIIb-IIIa antagonists[12]. The reason for this very surprising finding is unknown.

Platelets, as well as aggregating together following activation, can also interact with other cell types including blood leukocytes. Platelet–leukocyte conjugate formation is mediated mainly by P-selectin on platelets interacting with its ligand PSGL-1 on leukocytes[13,14]. Such conjugate formation is associated with leukocyte activation with increased expression of CD11b and also of tissue factor. The latter has the potential to support thrombus formation via activation of the coagulation cascade with enhanced thrombin generation and fibrin production[15,16]. Enhanced tissue factor expression has been seen in patients with acute vascular disease[17].

Here we measured the changes that occur in these parameters when blood is stirred with and without various platelet agonists and the way in which two GPIIb-IIIa antagonists abciximab and MK-852 modulate the results obtained. We also investigated how a combination of aspirin, dipyridamole and AR-C69931 (Asp/Dip/AR-C) affects the results. We used AR-C6993, a direct-acting $P2Y_{12}$ antagonist that targets the same ADP receptor as clopidogrel, since the latter is a pro-drug with no *in vitro* activity[18].

Materials and methods

Blood was drawn from healthy volunteers who denied drug intake during the previous 2 weeks. The blood was placed in polystyrene tubes containing hirudin as anticoagulant (Novartis, UK, final concentration 50 μg/ml) together with either saline (150 mmol/l NaCl as control), abciximab (c7E3, ReoPro, Centocor, UK, 10 and 20 μg/ml), MK-852 (Merck Sharp & Dohme, UK, 3, 10 and 30 μmol/l), or the combination of aspirin (Sigma, UK, 100 μmol/l), dipyridamole (Boehringer Ingelheim, Germany, 10 μmol/l) and AR-C69931 (AstraZeneca Charnwood, UK, 100 nmol/l). The blood was then kept at 37°C for 30 min and aliquots (480 μl) were then stirred at 1000 r.p.m. in a Multi-Sample Agitator (University of Nottingham, UK) for 2 min at 37°C. Either saline (20 μl, as control), ADP (Sigma, UK, 3 μmol/l), platelet activating factor (PAF, Sigma, UK, 1 μmol/l), adrenalin (Sigma, UK, 10 μmol/l), collagen (Nycomed, Germany, 2 μg/ml), or the combination of low concentration ADP (1 μmol/l), PAF (0.33 μmol/l) and adrenalin (3.33 μmol/l) were then added and the samples were stirred for a further 10 min.

Platelet–Leukocyte Conjugate Formation and Leukocyte CD11b Expression

50-μl aliquots of the blood were treated with 1.0 ml Erythrolyse solution (Serotec, UK) for 10 min at room temperature. The samples were then centrifuged at 380 *g* for 10 min and the pellets washed twice with FACSflow (Becton Dickinson, UK). The pellets were finally resuspended in 60 μl Dulbecco's PBS containing 10% (v/v) new born calf serum. A 30-μl aliquot was incubated at 4°C for 30 min in the dark with saturating concentrations of anti-CD14 : PE (Becton Dickinson, UK) to distinguish monocytes and neutrophils, and anti-CD42a : FITC (Serotec, UK) to identify platelets bound to monocytes and/or neutrophils. The platelet–monocyte and platelet–neutrophil conjugates were then quantitated by flow cytometry. A further 30-μl aliquot was incubated at 4°C for 30 min in the dark with anti-CD11b : FITC (Serotec, UK) to detect leukocyte activation. An isotype-matched IgG_1 : FITC (Serotec, UK) was used for non-specific membrane immunofluorescence.

Tissue Factor Expression on Monocytes and Neutrophils

50-μl aliquots of the blood were incubated at 4°C for 30 min in the dark with saturating anti-CD14 : PE and anti-TF : FITC (American Diagnostica Inc, UK) or isotype-matched mouse IgG_1 : FITC as a control. 1.0 ml Erythrolyse (10% v/v) was then added and the samples were left at room temperature for 10 min. The labelled samples were washed twice with 1.0 ml FACSflow and the pellet was resuspended in 500 μl fixing solution for flow cytometry.

Flow Cytometry

Platelet–leukocyte conjugates, and CD11b and surface tissue factor antigen expression on monocytes and neutrophils were quantified using a FACScan flow cytometer (Becton Dickinson, UK) equipped with a

5 W laser operating at 15 mW power and a wavelength of 488 nm, and connected to an Apple Mac G3 computer. Leukocytes were monitored using forward light scatter (an indicator of cell size), side light scatter (an indicator of cell complexity) and fluorescence. Linear modes were used for light scatter and log modes for fluorescence. A total of 10 000 leukocyte events were recorded for each sample. Monocytes were differentiated from other leukocytes by their CD14 : PE positivity. Platelet–leukocyte conjugates are reported as a percentage of CD42a : FITC positive leukocytes and also as the median CD42a fluorescence of the leukocyte populations. CD11b expression is reported as the median CD11b : FITC fluorescence of the leukocyte populations. Surface tissue factor expression on leukocytes is reported as a percentage of TF : FITC. The percentage of positive cells was obtained using a gate that divided the control IgG1 : FITC into 98% negative and 2% positive populations. The results obtained were in line with results obtained by other investigators[19–26] and with other analytical procedures, for example when leukocytes in whole blood were analysed directly without cell fixation, red cell lysis and centrifugation.

Statistical Analysis

Results are expressed as mean ± SEM. Data were compared using ANOVA and Dunnett's multiple comparison procedure (for comparisons of each active group versus control) or Bonferroni's procedure (for all pair-wise comparisons). Pearson bivariate correlation was used for analysis of associations. Data were analysed using SPSS (Microsoft Windows version 9).

Table 1. *Platelet–monocyte conjugate formation and monocyte activation in the presence of the various agonists and antagonists that were used. Results are mean ± SEM for 6–24 blood samples from different volunteers. Comparison by ANOVA with Dunnett's multiple comparison procedure*

	Control	Abciximab 10 μg/ml	Abciximab 20 μg/ml	MK-852 10 μmol/l	Asp/Dip/AR-C
P/M %					
unstirred	39 ± 5	31 ± 6	18 ± 2	33 ± 6	29 ± 5
saline	88 ± 3†	77 ± 4	71 ± 9	83 ± 5	73 ± 5
ADP	97 ± 1†	97 ± 1	91 ± 4	95 ± 1	84 ± 3*
collagen	99 ± 0†	99 ± 0	99 ± 0	99 ± 0	98 ± 0
PAF	99 ± 1†	n.e.	n.e.	98 ± 1	94 ± 2
adrenalin	96 ± 2†	n.e.	n.e.	80 ± 7	90 ± 4
ADP/PAF/Adr	98 ± 1†	n.e.	n.e.	97 ± 1	97 ± 1
P/M mf					
unstirred	7 ± 1	12 ± 1	3 ± 1	8 ± 1	10 ± 1
saline	31 ± 3	26 ± 4	26 ± 7	79 ± 22*	21 ± 4
ADP	66 ± 8†	217 ± 28*	154 ± 47*	338 ± 82	30 ± 5
collagen	181 ± 12†	840 ± 64*	500 ± 20*	610 ± 80*	146 ± 34
PAF	147 ± 16†	n.e.	n.e.	322 ± 71*	65 ± 11
adrenalin	50 ± 8	n.e.	n.e.	59 ± 32	25 ± 3
ADP/PAF/Adr	119 ± 13†	n.e.	n.e.	245 ± 57*	70 ± 10
M-CD11b					
unstirred	67 ± 7	72 ± 10	42 ± 2	66 ± 9	45 ± 4
saline	97 ± 8	99 ± 12	64 ± 5	104 ± 16	60 ± 7
ADP	107 ± 8†	121 ± 12	79 ± 10	127 ± 20	56 ± 5*
collagen	166 ± 10†	188 ± 12	148 ± 8	177 ± 15	103 ± 11*
PAF	157 ± 11†	n.e.	n.e.	123 ± 8	113 ± 11*
adrenalin	88 ± 10	n.e.	n.e.	48 ± 8	60 ± 8
ADP/PAF/Adr	133 ± 11†	n.e.	n.e.	102 ± 13	107 ± 12
M-TF %					
unstirred	6 ± 1	8 ± 2	6 ± 2	4 ± 1	5 ± 1
saline	12 ± 2	19 ± 5	21 ± 5	10 ± 2	10 ± 2
ADP	15 ± 3†	50 ± 9*	46 ± 11*	20 ± 5	11 ± 3
collagen	25 ± 3†	93 ± 2*	90 ± 3*	67 ± 7*	31 ± 5
PAF	17 ± 3†	n.e.	n.e.	41 ± 7*	16 ± 3
adrenalin	7 ± 2	n.e.	n.e.	12 ± 2	5 ± 1
ADP/PAF/Adr	16 ± 2	n.e.	n.e.	46 ± 7*	10 ± 2

P/M = platelet–monocyte conjugates; mf = median fluorescence; M-CD11b = CD11b (arbitrary units) on monocytes; M-TF = tissue factor on monocytes; Adr = adrenalin; Asp/Dip/AR-C = combination of aspirin, dipyridamole and AR-C69931; n.e. = no experiment performed

*$p < 0.01$ compared with control (no antagonist); †$p < 0.01$ compared with an unstirred sample (no agonist)

Results

Platelet–Monocyte Conjugate Formation and Monocyte Activation

Platelet–monocyte conjugate formation was determined directly by flow cytometry and the data are presented (Tables 1 and 2) either as the number of CD42a : FITC positive monocytes expressed as a percentage of the total number of monocyte present (P/M %), or as median CD42a fluorescence (P/M mf). The former presentation indicates the number of monocytes with platelets bound to them, and the latter provides an indication of the number of platelet bound to the monocytes. It can be seen that most monocytes bound platelets when stirred even without an agonist present but that the number of platelets that were bound increased according to the nature of the agonist used. Collagen and PAF were particularly effective. Platelet stimulation in the presence of a GPIIb-IIIa antagonist led to even higher values of P/M (mf) indicating that large numbers of platelets became bound to the monocytes under these conditions. The combination of Asp/Dip/AR-C did not enhance the platelet–monocyte interactions. An example of the dot plots obtained for monocytes from a single blood sample following stimulation with collagen in the absence and presence of abciximab is shown in Figure 1. The presence of platelets adherent to monocytes was confirmed by microscopy following fluorescence-activated cell sorting (Figure 2).

CD11b expression on monocytes increased when blood samples were stirred with an agonist (Table 1). In most cases the presence of abciximab or MK-852 did not affect this increase. The combination of Asp/Dip/AR-C significantly reduced the extent of the increased monocyte CD11b expression that occurred on stirring the blood samples with some of the agonists.

Stirring blood samples enhanced the amount of tissue factor on monocytes, particularly when collagen was also present (Tables 1 and 2). In the same way that abciximab and MK-852 had enhanced platelet–monocyte conjugate formation, these agents similarly enhanced surface tissue factor antigen on the monocytes. An example of the results obtained for monocytes from a single blood sample are shown in Figure 3. Figure 4 shows the relationship between the mean values obtained for P/M (mf) plotted against monocyte tissue factor expression (M-TF %) in the various experiments that were performed, and it can be seen that there is a correlation between the degree of conjugate formation and the degree of surface tissue factor expression on the monocytes. This emphasises that high levels of platelet–monocyte conjugate formation are associated with high levels of surface tissue factor antigen expression. Unlike the GPIIb-IIIa antagonists, the combination of Asp/Dip/AR-C did not increase tissue factor expression.

Platelet–Neutrophil Conjugate Formation and Neutrophil Activation

Platelet–neutrophil conjugate formation was also measured as P/N % and P/N mf (Table 3). Both measures revealed that platelet–neutrophil conjugate formation increased in response to the various agonists that were employed, although the changes were much less dramatic than was the case for platelet–monocyte

Table 2. *Platelet-monocyte conjugate formation and monocyte tissue factor expression in the presence of collagen and MK-852. Results are mean ± SEM for six blood samples from different volunteers. Comparison by paired* t*-test with Bonferroni correction*

	Control	MK-852 3 μmol/l	MK-852 10 μmol/l	MK-852 30 μmol/l
P/M %				
unstirred	31 ± 4	29 ± 3	32 ± 3	34 ± 3
saline	95 ± 2†	94 ± 2	92 ± 2	92 ± 4
collagen	98 ± 1†	98 ± 1	98 ± 1	98 ± 1
P/M mf				
unstirred	4 ± 1	3 ± 1	4 ± 1	3 ± 1
saline	41 ± 8	52 ± 10	47 ± 11	67 ± 20
collagen	163 ± 16†	712 ± 76*	548 ± 62*	553 ± 66*
M-TF %				
unstirred	3 ± 1	2 ± 1	3 ± 1	3 ± 1
saline	10 ± 2	16 ± 2	10 ± 2	13 ± 2
collagen	39 ± 4†	90 ± 3*	91 ± 3*	89 ± 2*

P/M = platelet–monocyte conjugates; mf = median fluorescence; M-TF = tissue factor on monocytes

*$p < 0.003$ compared with control (no antagonist); †$p < 0.005$ compared with an unstirred sample (no agonist)

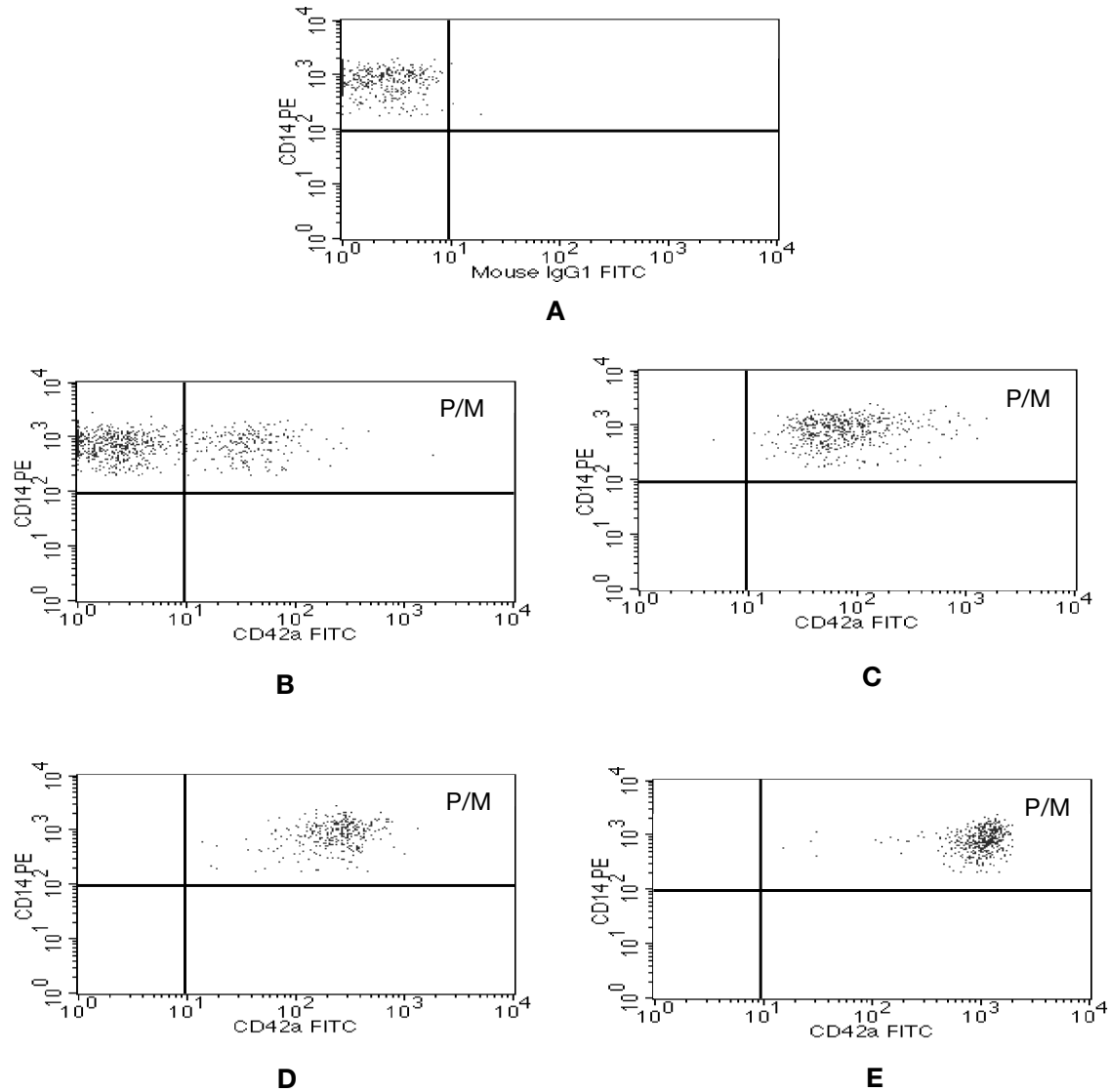

***Figure 1**. Flow cytometric analysis of platelet–monocyte conjugates according to CD42a positivity: A, isotype control; B, unstirred sample; C, stirred sample; D, collagen alone; E, collagen in the presence of 20 μg/ml abciximab*

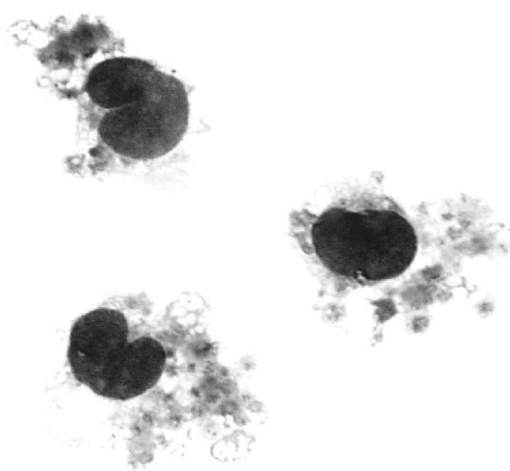

***Figure 2.** Monocytes with adherent platelets isolated following the addition of collagen to whole blood in the presence of 20 μg/ml abciximab. Monocytes were isolated using a Coulter EPICS Altra flow cytometer. The cells were sorted based on CD14/CD42a positivity. The laser configuration and optical filters were identical to those used in the FACScan*

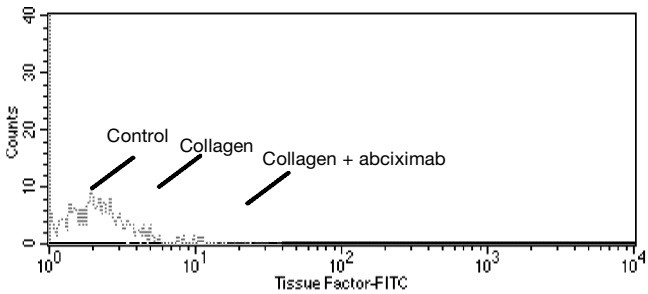

***Figure 3**. Overlaid histograms of tissue factor expression on the monocytes: isotype control, following collagen stimulation and following collagen stimulation in the presence of 20 μg/ml abciximab*

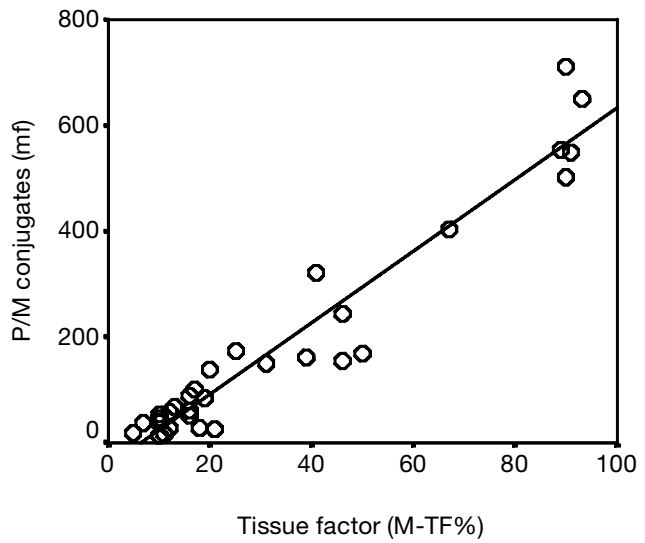

***Figure 4**. The correlation between the degree of platelet–monocyte conjugate formation (P/M mf) and the degree of tissue factor expression on the monocytes (M-TF%). $R^2 = 0.9$*

conjugates. In general, the various antagonists did not significantly affect the results obtained.

CD11b expression on neutrophils also tended to increase in response to stirring with the various agonists (Table 3), although the actual level of CD11b on neutrophils in unstirred blood was already very high compared with monocytes. Overall the various antagonists had little effect on neutrophil CD11b expression. There were no situations where an antagonist enhanced CD11b expression further than that obtained in response to the agonist. Tissue factor was not expressed on neutrophils and this was the situation whether or not platelet agonists or antagonists were present (data not shown).

Discussion

It is well known that stirring samples of whole blood results in some platelet activation resulting in some 'spontaneous' platelet aggregation[27]. Platelet agonists such as ADP, PAF, adrenalin and collagen increase the degree of platelet aggregation that occurs. It is also known that some platelet–leukocyte conjugate formation occurs following platelet activation, mainly via P-selectin on the surface of activated platelets and PSGL-1 on leukocytes. Conjugate formation can also be associated with leukocyte activation as judged by increased CD11b and also tissue factor[19,20,28]. Here we determined the effects of two GPIIb-IIIa antagonists on platelet–leukocyte interactions and compared the results with those obtained using a combination of Asp/Dip/AR-C.

Both abciximab and MK-852, which inhibit platelet aggregation by blocking fibrinogen binding to activated GPIIb-IIIa on the platelet surface, markedly enhanced platelet–monocyte conjugate formation. Particularly large increases in platelet–monocyte conjugate formation occurred following platelet stimulation with collagen, and the presence of platelets adherent to monocytes was confirmed by microscopy. Presumably the degree of platelet activation was such that the activated platelets bound avidly to the monocytes under the circumstances (in the presence of abciximab or MK-852) where platelet aggregation was inhibited. There was a trend towards less platelet binding to monocytes at the highest concentrations of abciximab and MK-852 that were used, and this may reflect some involvement of GPIIb-IIIa in platelet–leukocyte interactions, as reported by others[29,30]. Although GPIIb-IIIa antagonists markedly promoted adhesion of platelets to monocytes, adhesion of platelet to neutrophils was not enhanced.

Potentiation of platelet adhesion to monocytes by GPIIb-IIIa antagonists has been demonstrated before by some[21,23,31–33] but not all[22,34] authors and may depend on the degree of platelet activation and P-selectin expression that results. In our experiments platelet adhesion was most profound using collagen which is one of the stronger platelet agonists, and we have obtained very similar results using a thrombin receptor activating peptide (results not shown). Fredrickson *et al*. have also observed enhanced P-selectin-mediated leukocyte adhesion on a collagen-coated surface[35].

As well as promoting platelet adhesion to monocytes, the GPIIb-IIIa antagonists also promoted tissue factor exposure on the monocytes. The source of the increased levels of tissue factor on the monocytes is unknown, but it should be noted that the degree of tissue factor exposure obtained in the presence of the various agonists was found to correlate with the degree of platelet–monocyte conjugate formation that occurred. This may indicate that the platelets themselves are involved in promoting this monocyte tissue factor exposure. There is some evidence that platelets themselves contain tissue factor and possibly the interaction between platelets and monocytes serves to

Table 3. *Platelet-neutrophil conjugate formation and neutrophil activation in the presence of the various agonists and antagonists that were used. Results are mean ± SEM for 6–24 blood samples from different volunteers. Comparison by ANOVA with Dunnett's multiple comparison procedure*

	Control	Abciximab 10 μg/ml	Abciximab 20 μg/ml	MK-852 10 μmol/l	Asp/Dip/AR-C
P/N %					
unstirred	26 ± 4	33 ± 4	22 ± 4	33 ± 8	21 ± 5
saline	34 ± 6	32 ± 4	31 ± 6	32 ± 5	36 ± 6
ADP	50 ± 7	66 ± 5	52 ± 9	55 ± 8	40 ± 8
collagen	85 ± 2†	79 ± 3	74 ± 3	77 ± 6	60 ± 6*
PAF	65 ± 8†	n.e.	n.e.	59 ± 6	50 ± 8
adrenalin	49 ± 11	n.e.	n.e.	25 ± 7	32 ± 8
ADP/PAF/Adr	71 ± 8†	n.e.	n.e.	51 ± 11	48 ± 7
P/N mf					
unstirred	26 ± 2	27 ± 2	22 ± 2	27 ± 3	23 ± 2
saline	29 ± 3	28 ± 2	27 ± 3	26 ± 3	30 ± 3
ADP	40 ± 4	52 ± 5	40 ± 6	39 ± 6	33 ± 5
collagen	65 ± 5†	76 ± 7	65 ± 6	68 ± 8	43 ± 5
PAF	53 ± 6†	n.e.	n.e.	51 ± 8	39 ± 5
adrenalin	37 ± 4	n.e.	n.e.	23 ± 4	30 ± 3
ADP/PAF/Adr	53 ± 6†	n.e.	n.e.	45 ± 11	40 ± 5
N-CD11b					
unstirred	152 ± 10	160 ± 14	118 ± 13	161 ± 17	123 ± 10
saline	165 ± 11	178 ± 15	136 ± 15	177 ± 18	141 ± 13
ADP	174 ± 11	181 ± 13	140 ± 13	175 ± 17	137 ± 12
collagen	198 ± 10	185 ± 13	148 ± 10	188 ± 17	142 ± 12*
PAF	223 ± 17†	n.e.	n.e.	169 ± 11	201 ± 19
adrenalin	165 ± 16	n.e.	n.e.	112 ± 12	135 ± 17
ADP/PAF/Adr	205 ± 16	n.e.	n.e.	144 ± 15	188 ± 17

P/N = platelet–neutrophil conjugates; mf = median fluorescence; N-CD11b = CD11b (arbitrary units) on neutrophils; Adr = adrenalin; Asp/Dip/AR-C = combination of aspirin, dipyridamole and AR-C69931; n.e. = no experiment performed

*$p < 0.01$ compared with control (no antagonist); †$p < 0.01$ compared with an unstirred sample (no agonist)

expose this[25,36]. Enhanced platelet adhesion to monocytes in the presence of a GPIIb-IIIa antagonist did not result in enhanced CD11b expression which indicates that enhanced tissue factor antigen and CD11b expression occur via different mechanisms. Mickelson *et al*. detected either no enhancement or a reduction in CD11b by abciximab depending on the degree of leukocyte activation that occurred[34].

Our study has obvious limitations; in particular, experiments were performed wholly *in vitro* so that important modulators of cellular interactions were absent such as blood flow and vascular endothelium. Nevertheless, it is important to speculate on the possible consequences in man of enhanced exposure of tissue factor on monocytes following platelet activation in the presence of a GPIIb-IIIa antagonist. If the increased surface tissue factor antigen on monocytes was to contribute in an active way to thrombin formation and fibrin production, this might encourage thrombus growth despite inhibition of platelet aggregation. Further, fibrin clots are able to 'retract' in the presence of platelet aggregates, and enhanced clot formation in the absence of a mechanism leading to retraction of those clots could result in a situation that is worse than the normal pathological situation. These findings may be relevant to the failure of oral GPIIb-IIIa antagonists in recent large-scale clinical trials in patients with vascular disease[12]. It should be noted that the clinical success of these agents in the setting of percutaneous coronary interventions and acute ischaemic syndromes is under circumstances where heparin and other antithrombins are widely used in conjunction with the GPIIb-IIIa antagonists, which would have negated any hypercoagulation that may have resulted.

Finally, these experiments also compared the effects of the combination of Asp/Dip/AR-C on the various parameters that were measured. We have already reported that this combination of agents inhibits platelet aggregation and platelet–leukocyte conjugate formation in samples of whole blood stirred in the presence of ADP or PAF, and that the combination is more effective than the agents used individually or in pairs[37]. Here we show that, unlike GPIIb-IIIa antagonists, the combination did not enhance P/M induced by collagen and by ADP, PAF and adrenalin. They reduced monocyte activation as judged by CD11b expression. Unlike the GPIIb-IIIa antagonists, they did not promote

exposure of tissue factor antigen on monocytes. Presumably the different results obtained using this combination of well-established antiplatelet agents is a consequence of their ability to inhibit platelet activation rather than the interaction of platelets with each other once platelet activation has occurred[38].

Conclusions

Overall, we conclude that detailed examination of the effects of different antiplatelet agents on platelet–leukocyte interactions has revealed important differences in outcome that may be relevant to their clinical efficacy in patients with vascular disease. The potential for GPIIb-IIIa antagonists to promote the coagulation process following platelet activation, despite inhibiting platelet aggregation, may be the explanation of the negative clinical effects of these drugs. Our experiments show that a combination of antiplatelet agents, that collectively reduce platelet activation as well as platelet aggregation, does not provide such procoagulant potential.

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