ƒ ‹F | Œfia‹ ˙'e›ß• |–M^D‹ ª¤s›p e ·`⁄⁄¶i« ‡ł§i

ƒ ⁄p"O" ¤ ⁄£ƒP ‰Ł‡J¥ ⁄ ¸⁄"¥ † ›M"" ´ –· Q:⁄æ,ß§t

RGD ""peptides⁄ ¸§ beta3 integrin§ ¯Ø""‹ „ § ¤ ‹ ¡'°

(1/3)

›p e^ §¡G §O«‹›p e

›p e‰ ¸s„¡GNSC92-2320-B-038-026-

ıƒ ·`¶¡¡G92 ƒ~08⁄°01⁄ Ø 93 ƒ~07⁄°31⁄Ø

ıƒ ‡ ƒ ¡G» O ¥´ ˙⁄j ˙^ ‡z ˙‹

›p e¥ D ø⁄¦HG‡ \ •"_

‡ł§i^ «‹¡G" °†‡ł§i

‡B†z⁄ ¦Ł¡¡G¥»›p e¥ i ⁄ ‰ ¶‹d,

⁄⁄ ¥` Œ 93 ƒ~5⁄°31⁄Ø

ƒ ‹ F | Œfia‹ ˙'e›ß• |–M^D‹ ª¤s›p„"ƒ¤"G‡ł§i

ƒ ⁄ p " O " ¤ ⁄ £ ƒ P ‰ Ł ‡ ¥ :⁄ ¸⁄ æ"¥ß§t ›M"" ´

RGD ""peptide ⁄ ¸§ beta 3 integrin § ¯Ø""‹ „ § ¤ (‹1/¡3')°

›p„"^ § O ¡ G@‹flª¤«s›p„"

›p e ‰,:„NSC 92-2320-B-038-026

ıƒ · ›› 92 ƒ~8 ⁄°1 ⁄ ø 93 ƒ~7 ⁄°31 ⁄Ø

¥ D ø⁄:H‡ \ • ˙ "–––´

›p„" »P⁄H›ß¡G

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ıƒ "c: ¥ x ¥´ ˙⁄j ˙´ ˙‹ ª¤s'

⁄ ⁄ ¥`Œ⁄ E Q⁄ƒT~⁄ ›⁄°⁄ G Q⁄KØ‹ P·`⁄ ›

Abstract

Endothelial integrity is necessary for the normal functioning of blood vessels and for maintaining a non-thrombotic state, whereas endothelial damage induces platelet adhesion to subendothelial matrices (such as collagen) following by trigger platelet aggregation. When platelets are activated by agonists, platelets initially undergo shape change and subsequently express fibrinogen receptor (GP IIb/IIIa complex, $\alpha_{IIb}\beta_3$ integrin) on their membrane surface. Binding of fibrinogen to $\alpha_{IIb}\beta_3$ integrin appears to be the final common pathway for platelet aggregation. Nowadays, it is well established that the β_3 integrin recognition site on fibrinogen involves the Arg-Gly-Asp (RGD) sequence. When platelet are activated, the morphological changes accompanied by changes in many signal transductions including intracellular Ca^{+2} mobilization, protein phosphorylation, phosphoinositide metabolism, prostanoid formation, intracellular pH changes, free radical formation, and release of growth factors (such as PDGF) or cytokines etc. On the other hand, activated but not resting, platelets have been shown to adhere to intact endothelium, it has been suggested that platelet thrombi may also occur in the absence of endothelial cell denudation, particularly in the microvasculature. However, the pathways responsible for the interaction of platelets and the endothelium are not well characterized. The present study is designed to compare and characterize the differences of intracellular signal transductions while platelet adhesion to various extracellular matrices (ECMs; collagen type IV, fibrinogen). Furthermore, this study is also designed to further clarify the roles of the different receptors and cell associated-adhesive proteins involving in the interaction of activated platelets with extracellulat. In addition, we also compare the relative inhibitory activities of RGD-containing peptides (triflavin) with those of monoclonal antibodies against the β_3 integrin in these reactions. In this study, we found that ADP (10 μM)- or thrombin (0.5 U/ml)-activated platelets adhesion to immobilized fibrinogen (0.5 mg/ml) and collagen type IV (20 μg/ml) were more pronounced as compared with the resting platelets in a concentration- and time-dependent manners. Activated platelets adhesion to immobilized collagen type IV was shown more pronounced than fibrinogen. On the other hand, the maximal adhesion was reached about 60 min after activated platelets addition t to microplate wells pre-coated with fibrinogen or collagen. On the other hand, the RGD-containing peptide, triflavin (10 μg/ml), and anti-β_3 monoclonal antibody, Abciximab (20 μg/ml) significantly inhibited the adhesion of resting and activated platelets to fibrinogen or collagen. Triflavin was shown more potent than Abciximab at inhibiting this adhesion.

Keywords¡Gplatelet, extracellular matrix, endothelial cell, Arg-Gly-Asp-containing peptide, anti-β_3 integrin monoclonal antibody

⁄G¡B†‡Si⁄″fie

(⁄@) ‰t¥ »P¥ ‴″

¯ Ø ⁄ ″ ¥ ¿ – ` ƒ ″ ‴ ″ ″¥nonthrombogenic†‴fl S ' ° ¡ A ¥ S¥ƒ L‴k'M ƒ ⁄ p Ö'ƒ¸ †G⁄ ‴″¤ ¥ L ƒ ¤ ¥ ⁄ ˇ †¡ƒA†fS„ ‡¥H{•¶ H A¥Þ »nG⁄Oƒ†† }h‴″«O ¯ @ ¤ ¤ ƒ ⁄S}Ø ‴ Ö¸ƒ fƒ] ‡»Þ ″ ¥ † ⁄M † ƒ pG(1) ¤ ƒ†S „ª‴ ″ ⁄ ″ ¥† ›M (2)S†ƒ†† }h„ ₩ fiƒ ƒ ƒ‡‹ ⁄ˇ‴″ ¤ (anti thrombin III or activated protein C) (3) ¤ x ƒ„⁄ ⁄ˇ fiƒ ƒ ƒ‡l‴″† M S@¥(4) ƒ fi Œ • » ,¤‴t″‹¸⁄ˇ (ƒp´ ·«ƒ¤β-globulin plasminogen '¸ plasmin) ¥H⁄ (5)´ ` ƒ ‹ ¥N‹ ⁄ˇ‴″ fiƒ ƒ ƒ‡l } ˜ – .¡.C¡¥‡¥″~ ‴ ″ ⁄ ″ ¥†¥ »M˜ ' æ ¥ X ¥¤¤ ‴¤«‰Ł⁄¸‰ , `ƒ ⁄ p Ö ¶i ⁄ ˇ†ƒp prostacyclin (Moncada, 1982) ⁄¸nitric oxide (Radomski et al.,1987) ¥¡C

• ƒ ″ ¤ ¤ ¶fi•`‡¤ ˇof ″ ⁄ ″ ¥†S »M‴″¤ ¤ fl}¡ˆ¥a‡¥ • |^ n̄S¥X ƒ ″ ⁄″¥Lh‴″ ‰Ł† ¥ (matrix protein)¡Cƒ ″‴¶″ƒ¡‴¥ fl ‹ O¥ ' ‴‰‴″‴«†z'° ¶ ƒ¡`¡BSK« ⁄ ˇ†¡B† ' ¸fl €r‴″• P ¥¡ƒB‹y ° ⁄ O ·⁄Ł″S—¯ (ƒpshear stress)¡B ƒ †G⁄ ⁄ ¿@¥″` x T ¥H⁄ ¸ƒ b o‴¢ ˇ†⁄ ⁄qƒ ⁄ p Ö'¥¸ ƒ † ¥⁄' ˜ 'æ‴″⁄ , »^ fl ¥¥¡C„ ˚fl ƒ fiG(arterial thrombosis)ƒ ¤¥¡Aƒ ″‴¶″ƒ¡‴⁄¸ƒ ⁄ þO‴″‹ ⁄ˇ¥i»¡ S Œ Ł› »n‴″¤ ƒ ¡A¥Bƒ„¤ ‴ ¶¡ƒ„ƒXS@¡A⁄ @ ƒ ƒ‡l‴″† ¥˝¥i«P¤ˇ¥t⁄@ƒ ƒ‡l‴″‹ ⁄ˇ¡C

ƒb¥ ¿ ¹‴¥″†z „ ⁄U¡Aƒ ⁄ þO⁄£•|‴ (adhesion)¤ ¥ ¿ – ` ‴ ″ ƒ ″⁄″¥ † ›M ⁄W¡A £«D⁄ ″ ¥ † ¤»M ¤ fl}¡ˆæA„fi ƒ ⁄ p Ö•‴¶ ¤ ⁄ ¥ ⁄ Lh‴″ ' ‰⁄(basement membrane)⁄W¡A ' ‰⁄¥ D n‹¥Œ collagen, elastic tissue, proteoglycans 'M⁄@¤· non-collagenous glycoproteins ' ″cƒ¤(Yurchenco & Schittny, 1990)¡ F ¥«ecollagen ⁄ ₩ ƒ†14 ″ subtypes¡A‹ø¥i⁄ ‹ ¤ ⁄ ĵ : fibrillar collagen type I¡BII¡BIII 'MV¡F¤ I ' non-fibrillar collagens type¡F¤ ⁄⁄ fibrillar collagen type I ‹ ¯ Ø″›«›n‴″ collagen subtype¡ A ₩ ›fnsƒb' ¥ ‰S' ¸ƒ , x (tendon)¡Cƒbƒ ″⁄¥″ Dnfsƒb¥~...h¡Aƒb ⁄⁄...h⁄ ⁄″...h¥ ƒ†⁄ S}G¡F¥ƒ¥i¥H¥ ⁄ ″ ¥ † '›Þ¥ •˜ƒ † ›M' ƒXƒ¤¡Cƒ bo¥´ ˚fl ‴‹ w⁄ˇflg‴ ″ ƒ″(atherosclerotic vessel)⁄″...h¡A¥i o†{collagen type I ⁄j¶q‴″...W¥´¡F¥t ¥~¡Acollagen type IV ˜ ' non-fibrillar collagen¡Fcollagen type IV • »Þlaminin¡B heparin ¥H⁄¸heparan sulphate-containing proteoglycan ″cƒ¤⁄ ″ ¥ † ⁄S ' ‰⁄‴″ ¥D¯Ø(Akiyama et al.,1990)¡CCollagen ⁄£¶ ¨ £¤⁄ @ † ›M‴ ‴″⁄¶›–¡Aƒ Ł ¥ ¥ fl ‹ ⁄ˇƒ ⁄ þO⁄ _ƒ ⁄ þO˜ 'æ¤ ⁄″St‴«(α-granules '¸dense granules)'¸ _ƒ ⁄ þO fi¶ ⁄ ˇ†¡ C ¥¥¡Acollagen ¥ fl «P¤ˇ⁄@¤·† ›M¶ ƒ ...W¥´(proliferation)¡B⁄ ⁄ˇ (differentiation)⁄¸† ˚(migration)⁄ ˇ†(Cardarelli et al., 1992)¡Cƒ†^ collagen ¤ ¯Ø ‴″‹ ¤s¡A¥ «e⁄@fl ›{‹ ¥]St$\alpha_1\beta_1$¡B$\alpha_2\beta_1$ 'M$\alpha_3\beta_1$ ¥integrin¡C ƒ ⁄ þO‴ ¤ collagen fi¡A¥ fl ‹ O, g¥ ¥ƒ† ›M‰ ⁄W‴″$\alpha_2\beta_1$ integrin (glycoprotein Ia/IIa complex) (Saelman et al.,1994)¡CIntegrin ‴ ‹ ⁄@, s† ›M‴ ›–‴ † J ¥¤¯Ø¡A†o¤· integrin ƒb †″c⁄W'... ƒ „S¶¡†£«D–‹ ƒ ¡A†£Stƒ†¤ › ‹ †S‴″ƒ , †⁄¡Gα 'Mβ ƒ , †⁄¡, A„¤ ƒ , †⁄¥H «Dƒ @ ø`⁄Łƒ ¡ †¡C

ƒp«e›z¡A• ƒ ⁄ þO‴ ¤ ‰Ł† ¥ (ƒp collagen)«Æ¡A″ – • |⁄ _ƒ ⁄ þO˜ 'æ⁄ ˇ† o¥´¡Fƒ ⁄ þO⁄″St·X″ secretory granules (α-granules '¸dense granules)¡A • ƒ ⁄ þO¤ ¤ ,ß⁄p‴″¤ ¿E fi (ƒpADP)¡ A ˜ 'æ⁄ ˇ † ₩ ›d¥‴se-granules ‹ ¥D¡F › YD¤ ,ß⁄j‴″¤ ¿E⁄ ˇ† (ƒpthrombin '¸collagen) «h• |' ¯ê ª¶i⁄@¤B«P¤ˇα-granules ˜ 'æ¤ ⁄″St‴ «CDense-granules ¥ DnStƒ† serotonin, Ca^{+2} 'MADP¡Fƒ 脉α-granales ¥ Dn⁄″St¥H† ₩ ‰Ł‹ ¥Dƒp platelet factor-4¡Bβ-thrombospondin¡Bplatelet-derived

growth factor (PDGF)¡Bfibrinogen 'MvWF ¥ ¥ ¡ C ƒ b ˜ ' æ ⁄ ˇ † §«Æ¡Aƒ]ƒ ⁄p"O¤ ˜ ' æ " « " " ⁄ £ ƒ P ‹ ¡ ⁄ ˇ ⁄ ˇ † ¡ A " – ⁄ _ ƒ ⁄p"O fi¶ ⁄ˇ †¡C « Æ ¡ A • | ¤ ˇ – ф GM‰Hb/WIa" complex ($\alpha_{IIb}\beta_3$ integrin)† £ ¥ §¿"AW"§ ¯ ¡A ¤ ˇ ¤ƒ† ©⁄""⁄ @ ¤" † J ¥ ⁄⁄fibrinogen¡BvWF¡Bfibronectin¡Bvitronectin ¥ " ⁄W¥h⁄ _ ƒ ⁄ p Ö f(Plow et al., 1985)¡C†o¤ " † ¥ ⁄ _ " "fi¶ ⁄ˇ †A¥ D › n ‹ ⊕z„L¤l⁄ W" Arg-Gly-Asp (RGD)§ ƒC'MGP IIIa † ¥´ ƒX⁄ ˇ †¡Ho ¤ ⁄⁄¥Hfibrinogen § Œ " ‡ »«"¤ ƒ (Bennett, 1996)¡C

• ƒ " "¥† M¤¶¸¡ A ƒ p"Ö•|¤ subendothelial matrix ⁄ W A† ¥¤ƒ ⁄p " O ‹⁄¶i⁄@¤B⁄ o fi ¶ ˇ †Ö¥"¡ F £ƒ„§¥~¡A¥ « e ‹ ªs⁄w"⁄ ˇ " ¶ ⁄(p"O « ¥...‹ ¡ ⁄ ˇ ƒ⁄)•"|Ö‰– " ¤ ⁄ ¥ † › MW¥¡Af„• ¥ ƒ ⁄p"O⁄ _"¶fŒ (platelet thrombi) ƒb⁄ ¥ † ›M¤ §†¤ •l¶¸"™‹"A⁄ U ¥ ƒ¥idÖ¥´¡Afl §O‹ Ofb•L† ƒ " (microvasculature) " "æ (Tloti et al., 1991)¡C„ ¥ «e"™‹ ªsƒ ¤¥¡ A ƒ ⁄ p"O† ›M ‰⁄ W"¶¤·¤ ¯ Ø »P ¤ matrix protein ⁄W¥H⁄ ¿fpf⁄ _ ƒ ⁄ p " Ofi¶ ⁄ˇ †"" ´ †Q ªs"™‹ • ƒh¡F Mƒ ‹ ¡ ⁄ ˇ " ¶ ƒ fŒp"Ö ¤ ⁄ ¥ † ›M¥H⁄ ¤¿ † ›M¶ ¡ ƒ §Q¥¸ ¤ " ›⁄ W"ƒ " ¤ ¯Ø¥H⁄ „g¥ ƒ " " † ¥ • ... ¤ˇ¤ † ›M'..ƒ„¶ ¡ ‹‹" ¡Aƒ† ^ †o⁄ Ib–"™‹ ªs¤^£fh¡C¥ «eƒ†‹ ªs» ‹ ƒ ⁄ p " O † ›M‰P⁄sdW"¶in ¥H⁄¸ GP IIb/IIIa complex ($\alpha_{IIb}\beta_3$ integrin)ƒbƒ„" ˇ ⁄†Ib–§ Œt‹Y" flS'w¤ ƒ (Li et al., 1996)¡Af ' ¤ ¥ƒ" ¤ ¯ØƒpGP Ibα¡BGP IIa (collagen ¤ ¯Ø)¡BGP IV (thrombospondin ¤ ¯Ø)¡BGP IX ¥H⁄¸PECAM-1 (platelet-endothelial cell adhesion molecule-1)‹ O§_ƒ† »Pƒ„" ⁄ ˇ †¤¡^¥...†Q§ „ ¥ `A¡„F¥t⁄@⁄ Ib–¡A⁄ ¥ † M‰› ›–• ß ƒ " ¤ ¯Ø »P‹ ¡ ⁄ ˇ ƒ ⁄p"O™" ⁄ ˇ †¥¡A |¥..§ „ ¥ †M¡•Cƒ†‹ ªs† ‡ § ¡ ¥X¥ † › M W"™"Ø¤ † J ¥ƒpndothelial-sialylated glycoprotein¡BP-selectin ⁄¸ $\alpha_v\beta_3$ integrin ¥ fl »P¤ ⁄⁄(Buchanan et al., 1993)¡Fƒ ' E-selectin¡Bβ_1 integrin¡BPECAM-1¡BVCAM-1 (vascular cell adhesion molecule-1)¡BICAM-1 (intercellular adhesion melecule-1)⁄¸ GP Ibα‹ O§_ »P¤ ⁄⁄¡A«h¥ ⁄£† M¡¡C £⁄Fƒ p"O¸⁄ ¥ † M™" ›–" ⁄ ⁄l(ƒp ¤ ¯Ø) »Pƒ„¤ † M™" ⁄ ¥¬¡A' |» " † ¥ • ƒ„¤ † M"" ... ¡ A ' †‹ O¥ platelet-bound adhesive proteins ƒpfibrinogen¡Bfibronectin ⁄¸vWF »Pƒ„⁄ ˇ †¡'A¸ " ¥ endothelial cell-associated adhesive proteins ƒpcollagen type IV¡Bfibronectin 'M vWF ¤ • ‹ ¡ ⁄ ˇ ƒ⁄»P"O¥ † M⁄¶§¡"" ... ¡A¥ «e¤^⁄£† M¡¡A» ¶i⁄@¤ B ¿ª¤s ^ „Œ¡ C • ‹ ¡ ⁄ˇƒ"⁄p"O⁄ ¥ † › M « Æ A•⁄|¥ †_ › M " ¶"¡ƒ⁄˜¡A ⁄ ¥ † ›M⁄"‹ ^ T fi ¶ ·»..." ¸⁄⁄˜¡§¸¡A⁄·ƒ†‹ ªs"™"¯¶¡¡Fƒpƒ†› ¤· PKC isoforms †Q¡ ⁄ˇ¡BeNOS ⁄¸iNOS ‹ ¡ ⁄ˇ"Y t §'¡Be O§_• |«P¶i PDGF " " ˜'æ¸ prostacyclin (PGI_2) "™"ƒXƒ¤ ¥ ¥¡A¨–o§ › ¶i⁄@¤B¥h ƒß »P– ·Q¡C

† "æ« ƒ hQ" ‹ disintegrins "™"§ ƒ p"Ð Ðr† ¥ † ˜ †Q o†{(Gould et al., 1990)¡A†o¤· disintegrin ¤ amino acid § ƒC⁄Wƒ†⁄@ƒ ®q'°‹ ¤ ⁄ ⁄l a"æC " Þ†£ §tƒ†Arg-Gly-Asp (RGD)†o⁄ T amino acid ⁄ ⁄l¡A†oRGD ⁄ ⁄lƒPfi ⁄]‹ Ofibrinogen ƒX¤ ƒ ⁄p"ÖQIIbβ_3 integrin "™"†¡⁄ ¡ Œ]ƒ„¡A†o¤· disintegrin ¥i¥H'Mfibrinogen ˜v "§ƒ ⁄ p Ö⁄WÖQIIbβ_3 integrin ¤ ¯Ø¡ A]ƒ § ¤ ƒ ⁄ p " O ¶ ⁄Trifla¥Ö¡A⁄]‹ O ⁄@" disintegrin "™"† Ðr† ¥ ¡A- q¿Ø†£™" t ·"A(*Trimeresurus flavoviridis*)‹ ‡D⁄⁄' ⁄ ´ ¥X(Huang et al., 1991)¡C¥ „L¥h· X~™"‹ ªs †" G ª¥ ¡Atriflavin "™‰T‹ Of†... ⁄O "™"§ ƒ fiŒ" v ‡ ƒC

† "æ†o⁄T¡B¥|ƒ~§ › § † ⁄@..h™"– ·Q†o^ §t RGD "" $\alpha_{IIb}\beta_3$ integrin «œ§ fl•s "™"^ †z‹ ¡' ° ‡¥¸¡FƒpS › o† { triflavin ¥i¥H, g¥ § ¤ ⁄ ¥ † › MW"Ö$\alpha_v\beta_3$ integrin ¤ ¯Ø¶if § ¤ ⁄ ¥ † M™" »P´ † §@¥¸¡Ffb•s¥´ƒ " §@¥¸(angiogenesis)⁄ Ib–¡A

triflavin • | , g ¥ S ¤ ⁄ ″ ¥ † › M „ † › M ¥ ~ ‰ Ł ₫ ¥ ‟ ″ » P ´ †
† { ¥ X S ¤ • ¥ ˝ (Sheu et al., 1997a) ¡ © ƒ ″ ⁄ ¥ ¤ † › M l fi A ⁄ • | ¥ y › –
ƒ ¤ ƒ ⁄ p ‟ O fi ¶ ˇ ƒ † fi A Œ ⁄ ˇ † ¡ F ¥ t @ ⁄ Ł ƒ – ´ A ₫ O • ¶ ⁄ ¤ ‟ I ″ ƒ ″
† £ ¥ ″ ƒ ‹ ` Y ⁄ ˇ † ¡ ‟ A ″ , Y • ‹ † ¥ ƒ ¤ ƒ ″ j ¸ (angiospasm) ¡ C ‹ ª ¤ ¥ † þ triflavin ¥ i
ƒ fi ˜ ‟ ″ S ¤ ⁄ ƒ p ‟ ∅ ‟ ″ ¥ h ⁄ ″ ¥ ƒ ƒ ″ ‹ ` Y ˇ (Sheu et al., 1997b) ¡ C ƒ ƒ „ triflavin
⁄ ¶ ¨ „ ƒ fi Œ ƒ † ' œ ª ‟ ″ S ¤ S @ A ƒ P fi „ ƒ fi Œ ' æ H ″ ƒ ″ j ¸ S ¥ ⁄] ƒ † S ¤ S @
¥ ¸ ¡ ¥ t ¥ ~ ¡ A S › ¥ o † { triflavin ¥ i ' œ ª ‟ ″ S ¤ ¥ ƒ ' ° ¥ S U ⁄ _ ‟ ″ ƒ ⁄ þ O · ⁄
‟ ″ † { ¶ H (Sheu et al., 1999) ¡ ± ‟ æ ‟ ″ ‹ ª s S o † { triflavin ¥ i ' œ ª ‟ ″ S ¤ ƒ] fi † y X – i † N
' ⁄ _ ‟ ″ ƒ ″ ‰ ⁄ ‟ ″ ... W ¥ ˝ ⁄ ˇ † (Sheu et al., 2001) ¡ C ¥ ″ ″ ‟ ″ ‹ ª s ⁻ ª ¥ triflavin ¥ i
' œ ª ‟ ″ ´ _ ¯ Ø ″ ¥ β_3 integrin ' ⁄ _ ‟ ″ ƒ U ƒ fl e ƒ A ƒ ƒ „ ¥ i ¥ R ¥ S ¥ Q ¸ triflavin ‟ ″ fl S
' ° ¤ – • Q ‹ fl ª f ⁄ _ ‟ ″ ´ ⁄ ¸ RGD ^ ‟ « ‟ ″ ‹ „ ± fi ˜ ¡ ± t ¥ ~ ¡ A $\alpha_{IIb}\beta_3$ integrin ‟ ″
† ª S ⁻ Ø (Abciximab ¡ ± Reopro®) ¥ ⁄ w ƒ b ` { S ⁄ W ‹ ± fi ‟ v † y X – ‹ ± N _ ƒ ″ ⁄ ″
‰ ⁄ W ¥ ‟ ″ ƒ A ¶ ⁄ ˝ {¶ H (restenosis) (Simoons et al., 1994) ¡ C ¥ » „ Œ – • Q ƒ † ^ ƒ ⁄ p
‟ O ‟ ¤ ⁄ £ P ‟ ″ † › M ¥ ~ ‰ (± þ collagen type IV ⁄ ¸ fibrinogen) ' ⁄ _ ‟ ″ –
⁄ ˇ ⁄ fi ‡ S ' ° ¡ A ¥ ¸ ¥ H A , ƒ „ ⁄ G ″ ‟ † ¥ ⁄ „ þ O ‟ « Æ ‟ ″ fi ‡ S ' ° ⁄ ¸ › « › n ' ° ¡ C ¥ » » p
„ ″ S ' ¥ H ¿ ⁄ W › z ⊕ ⁄ G ‟ † J ¥ D n ‹ † ø G ″ ‟ † J ¥ ƒ ⁄ þ O ¶ £ ‟ ¡ B ¡
⁄ ˇ ⁄ ¸ fi ¶ ˇ ± fi ƒ S Œ ″ ‡ P ‟ ″ ¤ ƒ ¡ F ƒ p collagen type IV ‹ ƒ ⁄ þ O ‟ ¤ ⁄ ¥
⁄ U ‰ Ł ₫ ¥ ¥ ‟ Y fl ‟ † J ¥ A ƒ fibrinogen ‹ ⁄ _ ƒ ⁄ p ‟ O ¶ ‟ ″ › « ⊕ n ‟ † J
¥ ¡ C ‡ ¥ ~ S › – N ⁄ S S Q ¥ ¸ S t RGD ‟ ″ β_3 integrin « œ S fl (ƒ p triflavin) ' ¸ S β_3
integrin ‟ ″ † ª S ⁻ Ø (ƒ p anti-$\alpha_{IIb}\beta_3$ integrin, Abciximab) ¡ A ⁄ æ ß ⁄ W › z G ″ ^ ‟ « „ ƒ ⁄ p
‟ O ‟ ¤ ‰ Ł † J ¥ ‟ ″ S ° (potency ‟ ″ fi ‡ S ' °) ¡ C

(⁄ G) ‹ ª ¤ s ⁄ Ł ‟ k » P „ Œ … ˇ † ¸ › p ⁄ ‟ R ¡ G

(a) ¡ B fl ´ ⁄ ˇ † D ‹ r † J ¥ triflavin

† D r (*Trimeresurus flavoviridis*) – q R (Œ Latoxan) ` ° ⁄ J ¡ A ^ -2 ∅ ƒ S ƒ J b ⁄ ¡ C – N
Fractogel TSK HW-50 ¥ H »] ^ H ƒ A S ‟ ″ W H 1M NaCl ⁄ • ⊕ G fi S w ¡ A ¥ R ¶ ' 400 ml
‟ ″ … S R ″ ⁄ Ç – N D r ¥ ¸ 0.1 M NaCl • », ¡ A ƒ A , m ⁄ J ‰ ƒ Ø ⁄ W h ¡ A ¥ H 0.1 M NaCl • ¤ R
‹ y † G ¡ A b 278 nm ‟ i ‟ ± ⁄ U ƒ ‹ ¶ ⁄ ‟ ″ † D ″ ¤ ¥ ¡ C S Q ¥ ¸ † z ‟ R ‰ ⁄ ƒ † ″ R S @ ¥ ¸ ¡ A S Q
¥ ¸ ⁄ H ^ ¦ ¤ ‟ ″ fl ⁄ p ‟ Ø ¡ A ¥ ¿ ‹ ¶ ¤ ª ƒ † S ¤ ƒ ‟ ″ ⁄ p „ Ö ‹ C ¡ ' g S N › Æ fi Œ « Æ
ƒ H CM-Sephadex C-50 ´ ⁄ l ¥ « ‹ . W h h ‟ R k ¡ A ¥ H ⁄ T • ⊕ G ¶ i f ¤ R y .
(1) 0.05 M (pH 5.0) L ñ » 200 ml
(2) 0.05 M (pH 5.0) » P 0.2 M (pH 6.8) L ñ » ƒ U 450 ml
(3) 0.6 L ñ » ˇ (pH 8.0) 650 ml
ƒ ¶ ¤ R ⁄ U ‟ ″ ƒ U › ⁄ „ ″ (peak) ¡ A , ⊕ z ‟ R ¡ B S ¤ ƒ ⁄ p Ö ‹ ¡ B ª ¸ z ¿ g S N Æ fi
Œ ¶ A ƒ A ¥ ¸ Sephadex G-75 ‰ ƒ ‰ Ł L „ ´ b k ¥ H ″ » ˜ † B » ˇ (ammonium bicarbonate) • R ‹ y
† G ƒ ‹ ¶ ⁄ ¡ A ″ p « ⊕ z ‟ ″ ‹ « – S @ ¡ F « Æ ƒ H Sephadex G-50 ‰ ƒ ‰ Ł L „ ´ b k fl ⁄ ˇ ¡ A
› « ⁄ W › z ¤ B J ¡ C † « Æ – N ¥ b fl ⁄ ‟ ″ triflavin ¥ H reverse-phase HPLC ¤ fl ⁄ ˇ ¡ A ¥ H 1.0
ml/min ‟ ″ ‹ y † t ⁄ G • ⊕ G ‟ ″ ¤ R y – ¡ C • ⊕ G A: 100 % H_2O ¤ ″ S t 0.1% TFA
(trifluoroacetic acid) ¡ F • ⊕ G B: 20 % H_2O ¥ [80 % acetonitrile ¥ S t 0.1 % TFA ¡ C ƒ b
‹ ∅ 20 min † B ƒ ¶ † ⁄ @ ⁄ „ ″ ƒ S Y triflavin ¡ C S Q ¥ ¸ 20 % polyacrylamide gel ¶] „ b a S P
´ _ ¤ fl ´ « ¡ A ^ » P ⁄ W ⁄ l ¶ q ‟ ″ … – • ˙ « ⁄ œ e ⁄ ß ⁄ l ¶ q † ƒ b 7,500 ¥ ¥ k ¡ F ¤ ^ S ƒ ⁄ p
‟ O f ¶ ‹ ¦ ° ″ R ¡ A ⁄ ~ fl ‰ » f ¦ ⁄ ˇ ¥ X ‟ ″ triflavin ‹ ¦ ° ¤ S ¤ ¤ … ¥ C T

(b) ¡B»s†˘/H^ ƒ /p"O˜aflB†G

– N S ƒi fl acid/citrate/glucose (ACD)» P S ˜ @ •dƒ" ¥"Y"¥ : 9 (v/v)†V ƒ X¥A¥H 120 g ´ / 10 / ˜ ` ¡ C ¤ œ ¥ /W../p"Œ St.. (PRP),¥ {J heparin (6.4 U/ml), $PGE_1$ (0.5 µM) ' 37¢ J / ¥ ¯ fl0 / ˜`¡AƒA¥H 500 g ´ / 10 / ˜`¡A¥h/ Wh †G¡A¥¸ Tyrode •»†G¥ · · † F†¥«ÆTyrode •»†G–Nƒ /p"O¿ @ « ‰ $^a2\times10^9$ platelets/ml ¡C

(c) ¡Bƒ /p"O" ¤ † ›M¥~ ‰Ł†J¥ „Œ

– N / W › z » $ ˘S „ƒp"OS a fl ÐG¥Tyrode •»†G† M ‹ «Æ¡M¿ @ « ‰ ‹2 ¡ 10^9/ml ¡A– ¥ ¸ / £ ƒ P "‹ thrombin (0.5 U/ml) 'MADP (10 µM) ‹ ˘ƒ /p"O3 / ˜¡€N‹ ˘/ ¥…‹ ˘ƒ /p"O/ S O , mJ„coating / @h† ›M¥~ ‰Ł†J¥ fibrinogen (0.25-2.0 mg/ml)/¸collagen type IV (10-40 µg/ml) "/96-well i‰L /¡A/ SO/ ˘†10-120 / ˜`¡A/ ˘†† /F¥H tyrode •»†G† M/ G ƒ ¡F«Æ¥H•Ł‰L/ "R » / "Rƒ /p"O" ¤ / ƒP† ›M¥~ ‰Ł†J¥ "/"fi ¶¡-/ ˘†ƒ ‰u (Bellavite et al., 1994) ¡Fƒ ¥t/@† „Œ /¡A«h¥ –N‹ ˘' ¸¥…‹ ˘ƒ /p"O/ SO»P triflavin (10 µg/ml) ¥H/¸ anti-$\alpha_{IIb}\beta_3$ (20 µg/ml)† • S /Ø†10 / ˜`¡Aƒ A ƒ«ez¥[/ J w¥ coating / @h † ›M¥~ ‰Ł†J¥ "/"96-well •Ł‰ Ł// SO/ ˘†60 / ˜ ¥C¥»„Œ –N¥ i Ð–d(I) ƒ„/G " ^"%"¿ @ -/ ˘†ƒ ‰u¡A¤ ^ › p " ¥¤"‹ potency ¡F(II) ‹ ˘' ¸¥…‹ ˘ƒ /p"O " ¤ ƒ„/G" " †J¥ "/"fi ¶¡-/ ˘†ƒ ‰u¡A¥H¤ Ðo† A "/ ˘†fi ¶¡/¸ (III) ¥i`A , ƒ„/G" " †J¥ „ ƒ /p"O" fl /O"/"fi t S'¡C

(d) „Œ …˘ †¸›p/ "R¡G

„Œ …˘ ¥H„Œ † " G / S ›S(Mean) ± … – • »~f(S.E.M.) ¤ " ¥ ¡A––¤ † »P„Œ † /S¶ ¡ " "æ¥HStudent t-test S@†¸›p/ "R¡ ÐY $P < 0.05$ «h" ¥ ƒ † N,Y" fitSO¡C

(/T) †"G»P Q‰

Fig. 1A ¯ ª ¥ ¥ … ‹ ¡ / ˘ " " ƒ p"Coating'ƒ†fibrinogen (0.5 mg/ml) "/"microplate wells¡A S ¥˘ƒfb p"O ADP (10 µM)‹ ¡ >""" ‹ A/¡W† ƒ h]£†22% ¥ ¥k" " " †v¡ F˘ƒfbcoating ƒ†collagen type IV (20 µg/ml)"/"microplate well /W¡A /£‰ ƒ p"O S_ƒ†¤ ¤ ‹/¤" " †v'œ¯ ª"/æ fibrinogen ¤ ɵ (¥H 120 min ‹ ¤ ¡F¥ … ‹ ¡ / ƒ ¡A40.4 ¡ 3.5% vs. 30.4 ¡ 3.5%¡FADP ‹ ¡ / ˘ ƒ /¡P22.5 ¡ 2.3% vs. 40.5 ¡ 4.6%) (Fig. 1B) ¡A¥ ƒ ¥/i collagen type IV ,ß' " ¤ƒ/W¡A"Ð ‰ ƒ p"O†S_†Q‹ ˘¡F¥ t / @Ł¡A¥ Fig. 1 ¥ ¥ †QADP ‹ ¡ / ˘ " " ƒ ¤p†"Ø j/˘ † fi ¶¡ƒkø60 / ˜`¥ ¥k¡A S¤" "Ł/˘ † fi¶¡A¥ /£•|'œ¯ ª"ƒ A W¥"[†v(Fig. 1A and B) ¡C

ƒA" ¡A› ¥N‹ ˘ S ‹ ,ß–j" " ‹/˘ ƒ¡p thrombin (0.5 U/ml) ¡A¥ Fig. 2 ¥ ¥ ¡A /£‰ ¥ … ‹/"¸¤ thrombin ‹ ¡ / ˘ " " ƒ ¤p""O ¤ collagen type IV "/"¶q/@…¸/æ fibrinogen ¤ –ofh(¥H 120 min ‹ ¤ ¡F¥ … ‹ ¡ / ƒ ¡A43.9 ¡ 2.6% vs. 31.5 ¡ 3.4%¡F ‹ ¡ / ˘ ƒ /p¡A23.8 ¡ 3.5% vs. 42.6 ¡ 4.1%) ¡C" ƒX/W› z †"G¥ collagen type IV ƒb in vitro "/"" ‹ A/W3 fibrinogen fi e ' " ¤ ƒ /p"‰Ø W¡A¤ › ƒ]¥ifl »P fibrinogen ƒX¤ glycoprotein II_b/ III_a complex /Wfi ¡A›n stirring ƒ†^ ¡C

ƒA" ›Y¥H fl¶q/˘ †ƒ – ‰ u › p "«hY coating fibrinogen "/"¿ @ ¥ 0.25-2.0

mg/ml fi ¡ A § › o † { ¤ ¤ „ F ‡ ⁄ j 0.5-1.0 mg/ml ¡ A § « ¥ ¿ ø ß b...W ¥ ƒ 2.0 mg/ml ¥ ⁄ £ |..ƒ A ¥ [adhesion (Fig. 3) ¡ A › Y − N ƒ ⁄ p Ö ¤ RGD › P § t peptide, triflavin (10 µg/ml) ' ¿ anti-$\alpha_{IIb}\beta_3$ " " ‡ • § Abciximab (20 µg/ml) ⁄ ˘ ‡ ¡ A ¥ [⁄ J coating fibrinogen " " wells ⁄ ⁄ ¡ A § › triflavin ' M Abciximab ¤ " ƒ ¥ i ¥ H ' œ ª " " § ¤ ‡ ADP ‹ ¡ ˘ " " ƒ p " Ö ¤ fibrinogen (Fig. 3) ¡ C æ, ß " " " potency ¡ A « h ϕ { triflavin § ¤ fi " G ⁄ æ Abciximab ƒ n ¢ ¤ (9.5 ¡ 1.9% vs 14.6 ¡ 1.2% ¡ A ¥ H fibrinogen 0.5 mg/ml ‹ ¤) ¡ A ƒ „ ¡ " ¥ p ¥ X † ƒ b coating ƒ ‡ collagen type IV " " wells ⁄ " F ¥ Fig. 4 ⁄ ⁄ ¥ i " collagen ‡ ⁄ j " " " ¿ 20 µg/ml ¥ ¥ k ¡ A ƒ triflavin (10 µg/ml) " " § ¤ fi " G « h ' œ ª " " ⁄ æ Abciximab − j ‡ ƒ (Fig. 4) ¡ A › ƒ] ¥ i fl » P triflavin „ receptor " " ¿ ¸ ' M ⁄ O ⁄ æ Abciximab ¤ − o − j ¡ F ⁄ æ ß Abciximab „ § ¤ ƒ p " Ö ¤ fibrinogen ⁄ ¿ collagen type IV ¥ i œ ª o † ¡ A Abciximab § ¤ ƒ p " Ö ¤ fibrinogen ⁄ æ collagen type IV ' œ ª ¡ A › ƒ] ‹ fibrinogen " ¤ ƒ p " Ö › M ‰ ⁄ ¥ › n ‹ O ´ ˙ GP II_b/ III_a complex ($\alpha_{IIb}\beta_3$ integrin) ¡ A ƒ collagen ¥ D › n O ¥ ˙ GP II_a receptor ¡ A ƒ] ƒ „ Abciximab , ß ' § ¤ fibrinogen " " " ⁄ ¡ C ‡

⁄ T ¡ B ƒ ⁄ ˜ m

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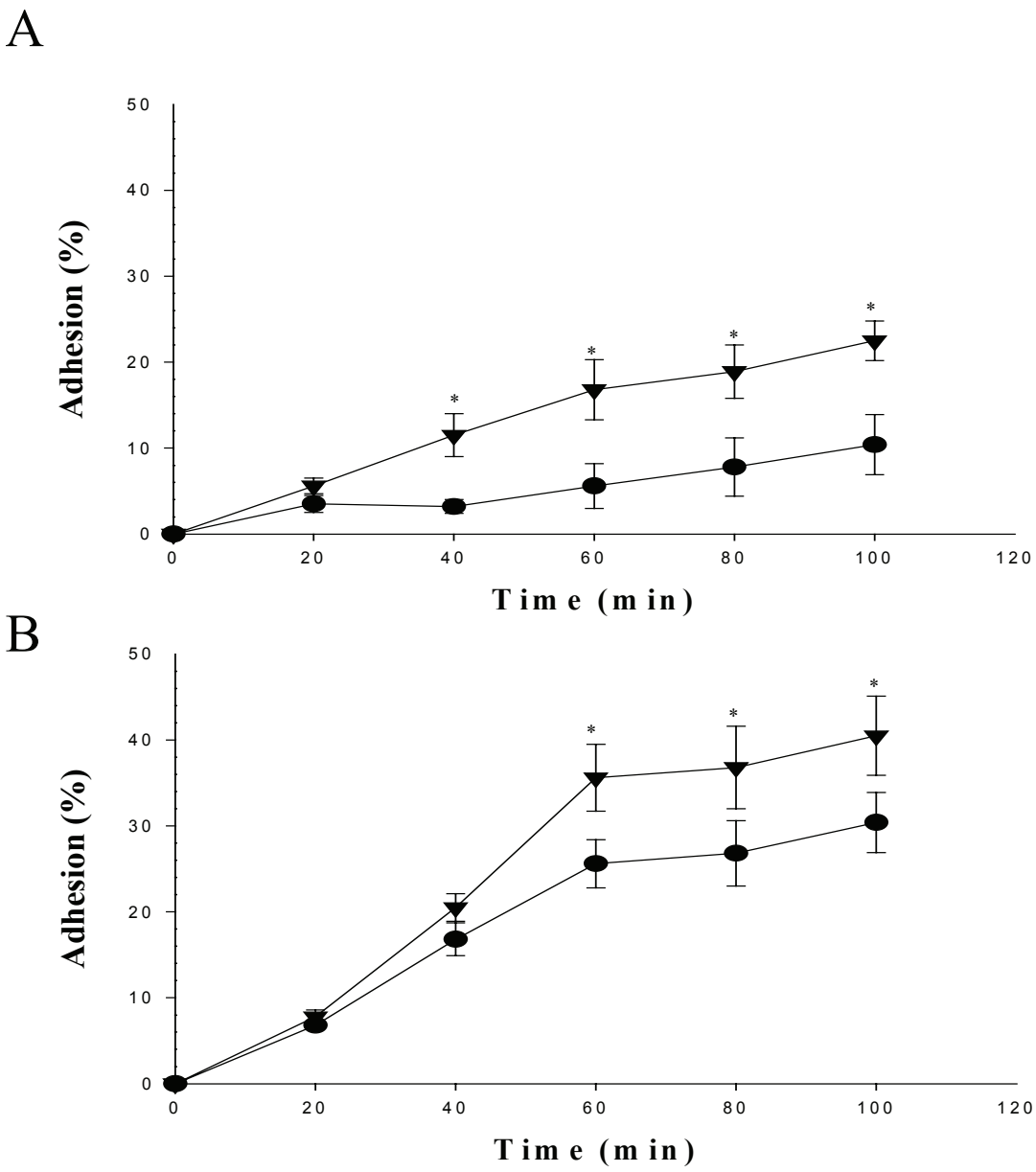

Fig. 1 Time courses of adhesion of unstimulated and ADP-stimulated platelets to immobilized fibrinogen and collagen type IV. Washed platelets were incubated at the indicated time (0-120 min) in the absence (¡·) or presence (¡¿) of ADP (10 μM) in microplate wells pre-coated with (A) fibrinogen (0.5 mg/ml) and (B) collagen type IV (20 μg/ml). Data are presented as means ¡ S.E.M. (n=4). *P < 0.05 as compared with the control group (in the absence of ADP).

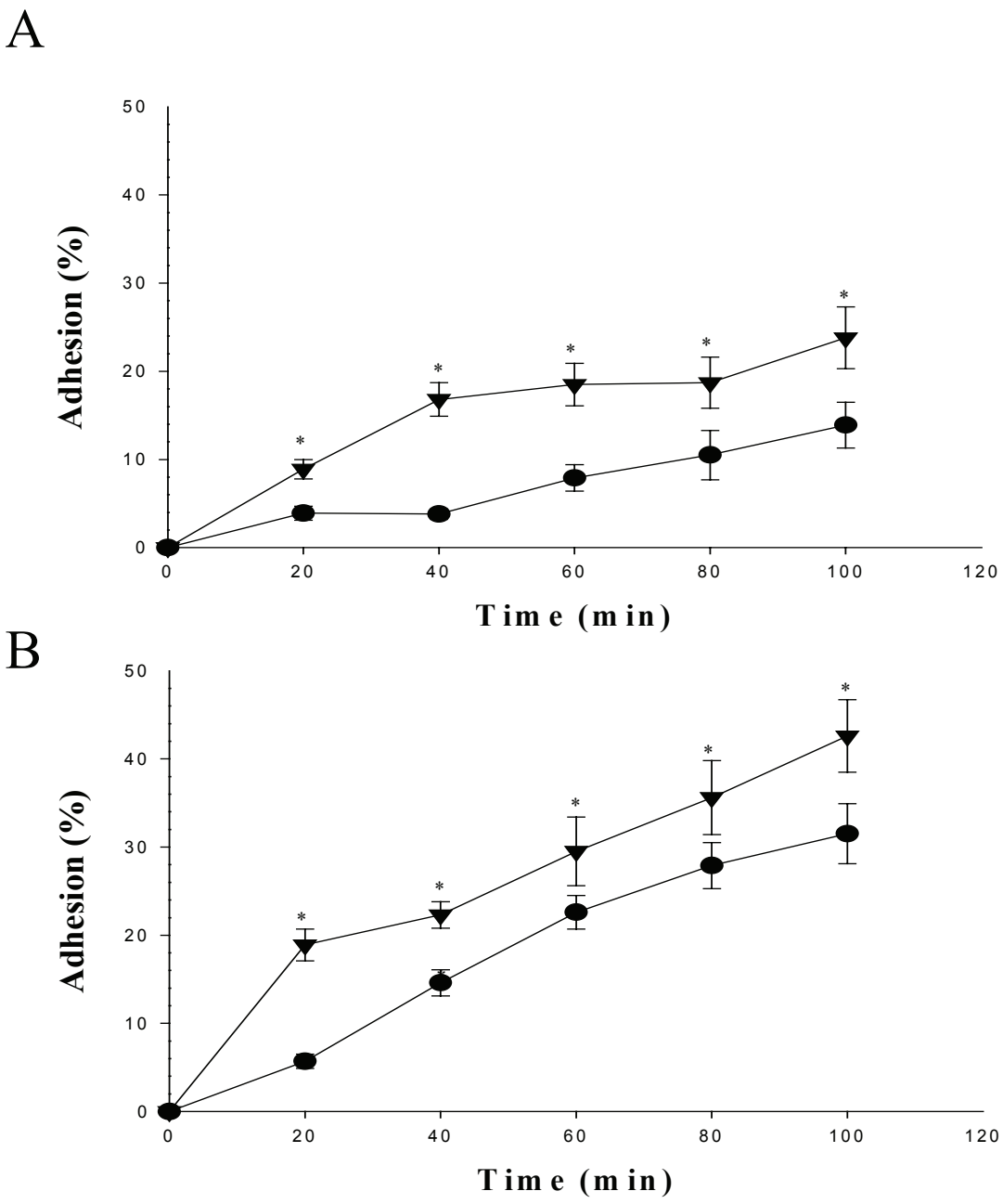

Fig. 2 Time courses of adhesion of unstimulated and thrombin-stimulated platelets to immobilized fibrinogen and collagen type IV. Washed platelets were incubated at the indicated time (0-120 min) in the absence (¡ ·) or presence (¡ ¿) of thrombin (0.5 U/ml) in microplate wells pre-coated with (A) fibrinogen (0.5 mg/ml) and (B) collagen type IV (20 µg/ml). Data are presented as means ¡ S.E.M. (n=4). *$P < 0.05$ as compared with the control group (in the absence of thrombin).

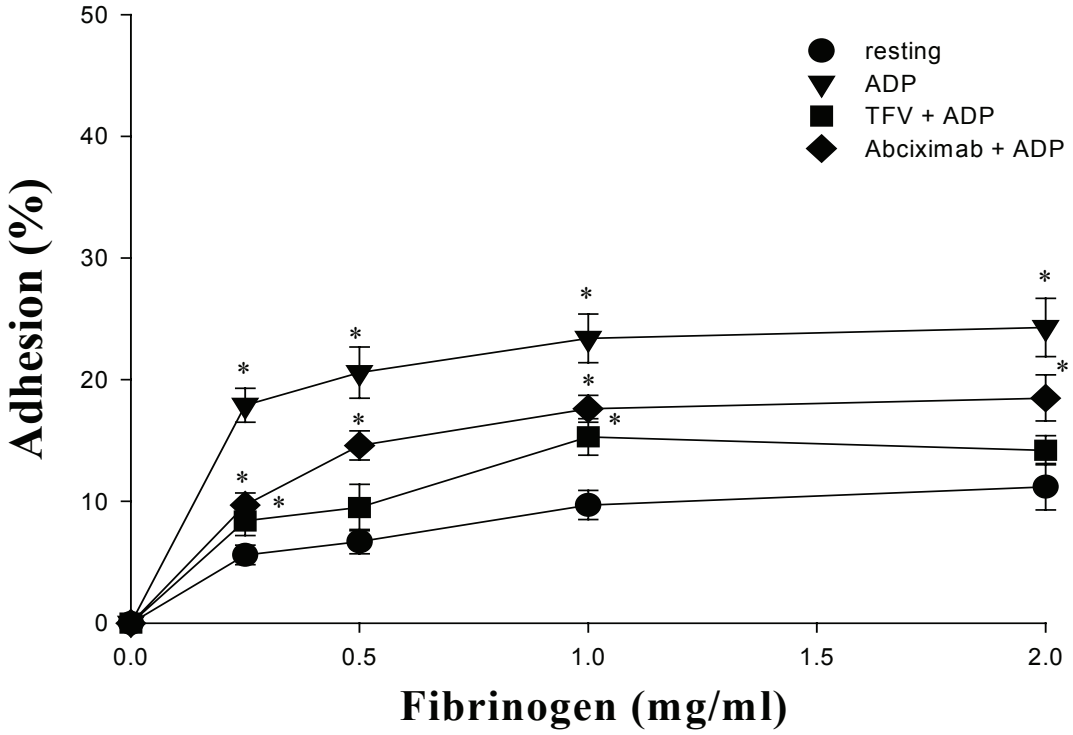

Fig. 3 Concentration-response courses of adhesion of unstimulated and ADP-stimulated platelets to immobilized fibrinogen. Unstimulated (¡ ·) and ADP (10 µM)-stimulated platelets (¡ ¿) were incubated with various concentrations of immobilized fibrinogen (0.25-2.0 mg/ml) for 60 min in the absence or presence of triflavin (¡ ‰ TFV, 10 µg/ml) or Abciximab (¡ » 20 µg/ml) in microplate wells. Data are presented as means ¡ S.E.M. (n=4). **P* < 0.05 as compared with the control group (in the absence of ADP).

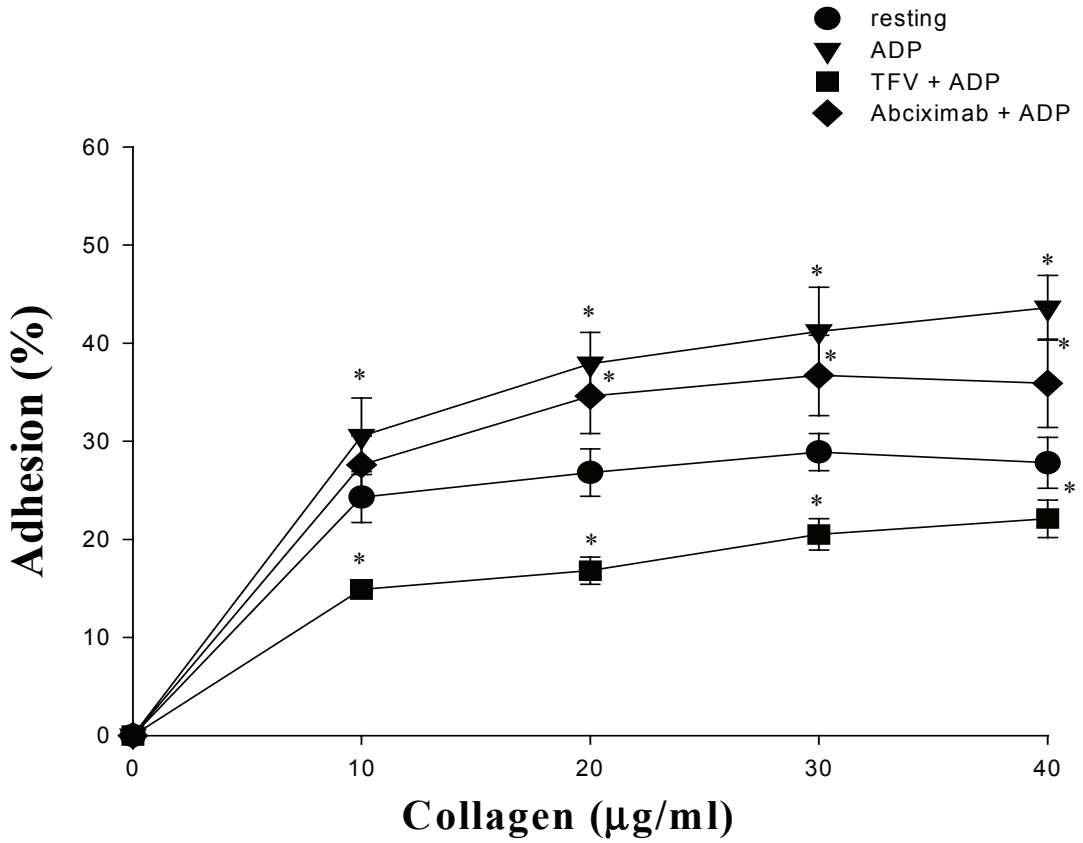

Fig. 4 Concentration-response courses of adhesion of unstimulated and ADP-stimulated platelets to immobilized collagen. Unstimulated (¡ ·) and ADP (10 µM)-stimulated platelets (¡ ¿) were incubated with various concentrations of immobilized collagen type IV (10-40 µg/ml) for 60 min in the absence or presence of triflavin (¡ ‰ TFV, 10 µg/ml) or Abciximab (¡ » 20 µg/ml) in microplate wells. Data are presented as means ¡ S.E.M. (n=4). **P* < 0.05 as compared with the control group (in the absence of ADP).