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Genistein, a Protein Tyrosine Kinase Inhibitor, Inhibits Thromboxane A_2-Mediated Human Platelet Responses

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SUMMARY

An isoflavone compound, genistein, which is known as a protein tyrosine kinase inhibitor, concentration-dependently (0.1–30 μg/ml) suppressed human platelet aggregation, serotonin secretion, and protein tyrosine phosphorylation induced by collagen or stable thromboxane A_2 analogs [U46619 and 9,11-epithio-11,12-methano-thromboxane A_2 (STA_2)]. However, genistein did not inhibit these thrombin (0.1 unit/ml)-induced platelet responses. Although thrombin induced an increase in the platelet phosphotyrosine content, genistein at 100 μg/ml only slightly attenuated thrombin-induced protein tyrosine phosphorylation. Genistein competitively inhibited [^{3}H]U46619 binding to washed platelets, in a concentration-dependent fashion. Daidzein (another isoflavone compound), which does not have a hydroxyl group at the 5-position of genistein and lacks inhibitory activity for protein tyrosine kinase, was found to suppress [^{3}H]U46619 binding, leading to the inhibition of collagen- or STA_2-induced platelet responses. These results indicate that the blockage by genistein of platelet responses induced by collagen or thromboxane A_2 is due to its preventive action on thromboxane A_2 binding to the receptor, rather than via inhibition of protein tyrosine phosphorylation, and that the drug does not appear to be a particularly good inhibitor of tyrosine phosphorylation in intact platelets.

Protein tyrosine kinases are distributed in a wide variety of cells. It is assumed that the phosphorylation of proteins at tyrosine residues plays an important role in transmembrane signaling for cell proliferation and transformation (1). However, it should be noted that protein tyrosine kinase activity is unusually abundant in terminally differentiated platelets (2, 3) but its physiological significance has remained unknown.

The initial biochemical response of platelets to various agonists is activation of phospholipase C, which cleaves PIP_2, leading to generation of the second messengers 1,2-DG and inositol 1,4,5-trisphosphate. The involvement of a GTP-binding protein, putatively but as yet unidentified as Gp, in phospholipase C regulation is thought to be likely (4). Recently, the possibility was presented that, in 3T3 mouse fibroblasts (5) and A431 cells (5, 6), protein tyrosine phosphorylation of γ-type phospholipase C by PDGF receptor or EGF receptor kinases may cause its activation. The association of phosphatidylinositol 3-kinase activity with protein tyrosine kinase (7) is another example of a link with protein tyrosine kinase in the signal transduction system. Human platelets contain extremely high levels of $pp60^{c\text{-}src}$, and protein tyrosine phosphorylation is transiently increased after thrombin stimulation (8–10). The biological functions of $pp60^{c\text{-}src}$ and protein tyrosine phosphorylation in platelets are unclear. However, these observations suggest the possible involvement of protein tyrosine kinases in platelet signal transduction.

The identity and functions of protein tyrosine-phosphorylated proteins are not well described. Thus, the present experiments were undertaken to gain more insight into the functional roles of protein tyrosine phosphorylation in platelet signal transduction, by use of a potent inhibitor of protein tyrosine kinase, genistein, which is an isoflavone compound from fermentation broth of *Pseudomonas spp.* (11). This drug was found to inhibit collagen- and TXA_2-induced platelet activation, whereas it exerted little effect on thrombin-mediated platelet responses. However, surprisingly, another isoflavone compound, daidzein, which has no inhibitory activity for protein tyrosine kinase (11), also suppressed platelet responses elicited by collagen or TXA_2, suggesting that the inhibition by genistein is not caused by attenuated protein tyrosine phosphorylation but is due to inhibition of other site(s).

Materials and Methods

Isolation of platelets. Blood was obtained from healthy volunteers who had not taken any drugs for the previous 2 weeks. The entire

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ABBREVIATIONS: PIP_2, phosphatidylinositol 4,5-bisphosphate; TXA_2, thromboxane A_2; STA_2, 9,11-epithio-11,12-methano-thromboxane A_2; U46619, 9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$; ONO-3708, (9,10),(11,12)-dideoxy-9α,11α-dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclopentyl-16,17,18,19,20-petanor-15-epi-thromboxane A_2; PA, phosphatidic acid; 1,2-DG, 1,2-diacylglycerol; BSA, bovine serum albumin; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; PRP, platelet-rich plasma; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; PAF, platelet-activating factor.

platelet isolation procedure was performed at room temperature. The blood was centrifuged at 160 × *g* for 10 min, and the PRP was collected. In some experiments, platelets were labeled with [^{3}H]arachidonic acid (0.2 μCi/ml) or [^{14}C]serotonin (0.01 μCi/ml) at room temperature for 60 min. PRP, containing 0.5 unit/ml potato apyrase and 0.2 μg/ml prostaglandin I_2, was gently layered on a 40% (w/v) BSA solution and then centrifuged at 1500 × *g* for 10 min. The sedimented platelets were gently resuspended in Tyrode-HEPES buffer (134 mM NaCl, 12 mM $NaHCO_3$, 2.9 mM KCl, 0.36 mM NaH_2PO_4, 1 mM $MgCl_2$, 5.6 mM dextrose, 0.1% BSA, 10 mM HEPES, pH 7.40). Platelets were then filtered through a Sepharose 2B column equilibrated with Tyrode-HEPES buffer.

Platelet aggregation and serotonin secretion. Platelets prepared as above were diluted, to make a suspension of 10^9 cells/ml, and supplemented with 1 mM $CaCl_2$ (final concentration). Platelet aggregation studies were carried out by recording light transmission through samples of platelet suspension stirred at 1100 rpm in a cuvett in an aggregometer (Bryston Manufacturing Ltd.). [^{14}C]Serotonin release was measured as described previously (12).

Lipid extraction and analysis. [^{3}H]Arachidonic acid-labeled platelets were suspended in Tyrode-HEPES buffer at a concentration of 1 × 10^9 cells/ml, and the final Ca^{2+} concentration was adjusted to 1 mM using 200 mM $CaCl_2$. Platelets (0.5 ml) were preincubated for appropriate times with genistein before addition of agonists. The reactions were terminated by addition of 2.0 ml of chloroform/methanol (1:2, v/v). The two phases were separated by the addition of 0.6 ml of chloroform and 0.6 ml of a 0.2 M KCl/5 mM EDTA mixture (13). After centrifugation at 1500 × *g* for 5 min, the organic phase was collected, dried under nitrogen gas, and resuspended in a small volume of chloroform/methanol (6:1, v/v). Neutral lipids were separated on silica gel 60 plates impregnated with 0.4 M boric acid, using a solvent system of chloroform/acetone (96:4, v/v). Phospholipids were separated by two-dimensional thin layer chromatography on silica gel 60 plates, using chloroform/methanol/13.5 M NH_3 (65:35:6, v/v) in the first dimension and chloroform/acetone/methanol/acetic acid/water (30:40:10:10:5, v/v) in the second dimension (13). Spots were identified by comigration with authentic standards. The radioactivity in the individual spots was measured using a liquid scintillation counter.

Antiphosphotyrosine immunoblot assay. Platelets were solubilized in Laemmli sample buffer (14) and boiled for 3 min. Platelet proteins were separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride membrane (GVHP filter). Immunoblot assays were performed as described (15), with slight modifications (16). The membrane was soaked overnight in blotting buffer, 3% ovalbumin in Tris-buffered saline (10 mM Tris, pH 7.40, 150 mM NaCl, 0.05% Tween 20, 0.02% NaN_3), and washed in Tris-buffered saline. The membrane was then incubated with antiphosphotyrosine antibodies, followed by ^{125}I-Protein A. The radioactive bands were visualized by autoradiography.

[^{3}H]U46619 binding assay. Binding assays were carried out according to the method of Kattelman *et al.* (17), with slight modification. Assay buffer (138 mM NaCl, 5 mM KCl, 5.6 mM dextrose, 25 mM Tris·HCl, pH 7.40) containing 1 mM aspirin and 0.1% BSA was used instead of Tyrode-HEPES buffer during platelet preparation, and platelets were suspended in the same buffer at a concentration of 4 × 10^8 cells/ml. Aliquots of platelet suspension (0.8 ml) were diluted to a final volume of 1.0 ml, in the presence of 8 nM [^{3}H]U46619 (0.1 μCi/assay) and the drug, and then incubated at 37° for 30 min. The reaction was terminated by the addition of 5 ml of ice-cold assay buffer. The platelet suspensions were rapidly suctioned through premoistened GF/C filters. The filters were washed three times with 5 ml of ice-cold assay buffer and air dried. The radioactivity retained on the filters was measured in a scintillation counter. Specific binding was assessed from the difference between binding in the absence and presence of excess (1000-fold) unlabeled U46619.

Materials. [5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid (190.4 Ci/mmol), 5-hydroxy[^{14}C]tryptamine (serotonin) (55 mCi/mmol), and [^{3}H]U46619 (12.1 Ci/mmol) were purchased from New England Nuclear. Collagen was obtained from Hormon-Chemie. U46619 was from Cayman Chemical Co. Sepharose 2B was from Pharmacia. Silica gel 60 plates were from Merck, and GF/C glass fiber filters were from Whatman. GVHP filters were from Millipore. STA_2 and ONO 3708 were provided by Ono Pharmaceutical Co. Genistein was generously given by Dr. Hiroshi Ogawara (Meiji College of Pharmacy, Tokyo). Herbimycin A was a generous gift from Dr. Yoshimasa Uehara (National Institute of Health, Tokyo), and ST638 was from Dr. Tadayoshi Shiraishi (Kanegafuchi Chemical Industry, Takasago, Japan). Daidzein was purchased from Extrasynthese. All other chemicals were of analytical grade.

Results

Effect of genistein on platelet aggregation. Gel-filtered platelets were preincubated for 5 min with a protein tyrosine kinase inhibitor and then stimulated by agonists. Genistein (10 μg/ml) almost completely prevented platelet aggregation induced by collagen (1 μg/ml) and the stable TXA_2 receptor agonist STA_2 (0.1 μM) (Fig. 1A). The effect of U46619, another TXA_2 receptor agonist, on platelet aggregation was also abolished (data not shown). In contrast, genistein at up to 100 μg/ml had no significant effects on thrombin (0.1 unit/ml)-induced platelet aggregation.

Genistein suppressed STA_2- and collagen-evoked platelet

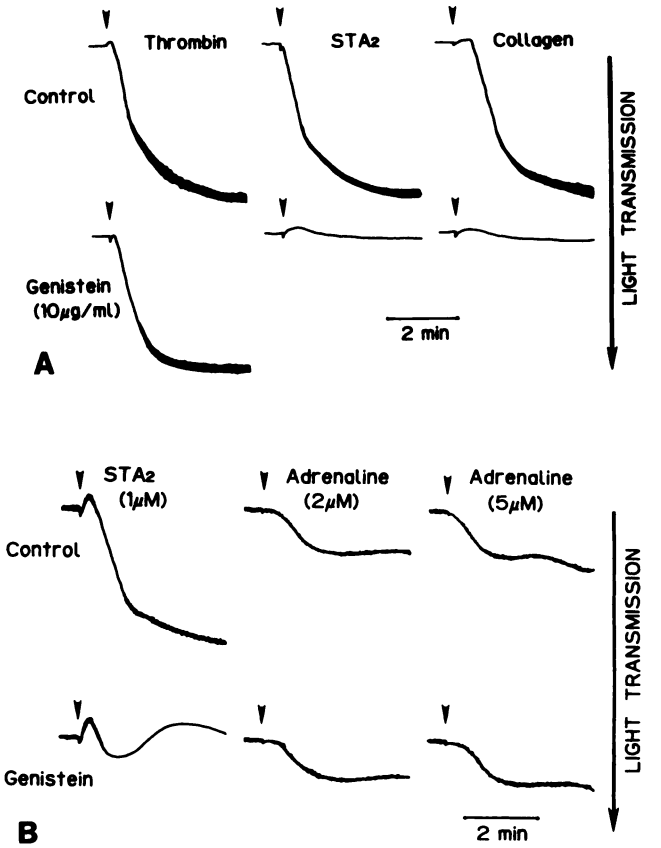

Fig. 1. Effects of genistein on human platelet aggregation. A, Washed human platelets were incubated at 37° for 5 min with buffer (*upper traces*) or genistein (10 μg/ml) (*lower traces*) and then stimulated (*arrowhead*) with 0.1 unit/ml thrombin (*left traces*), 0.1 μM STA_2 (*middle traces*), or 1 μg/ml collagen (*right traces*). B, Aspirin (100 μM)-treated PRP was incubated at 37° for 5 min with buffer (*upper traces*) or genistein (10 μg/ml) (*lower traces*) and then stimulated (*arrowhead*) with 1 μM STA_2 or 2 or 5 μM adrenaline. The results are shown as typical traces from one experiment, which is representative of three others.

aggregation in PRP. However, the drug had no inhibitory effect on the primary aggregation stimulated by adrenaline in aspirin (100 μM)-treated PRP (Fig. 1B). Moreover, the primary aggregation induced by ADP was not prevented by genistein (data not shown).

Effect of genistein on serotonin secretion. The effect of genistein on platelet [^{14}C]serotonin release is shown in Fig. 2. The reactions were terminated 60 sec after stimulation. The STA_2-induced release reaction was concentration-dependently inhibited, and complete suppression was noted at 30 μg/ml concentrations of the drug. The IC_{50} for 0.1 μM STA_2-induced secretion was approximately 1 μg/ml. Genistein also suppressed serotonin release stimulated by collagen and U46619, in a concentration-dependent fashion (data not shown). The drug had no effect on thrombin (0.1 unit/ml) stimulation.

Effect of genistein on phospholipid turnover. Platelet activation is thought to be closely coupled with phosphoinositide turnover, which generates the second messenger molecules 1,2-DG and inositol 1,4,5-trisphosphate. We have shown the presence of phospholipase C-γ_2 and purified it to homogeneity (18). It is also suggested that phosphorylation of phospholipase C-γ on tyrosine residues, after stimulation of the PDGF and EGF receptors, may cause its activation in 3T3 fibroblasts and A431 cells, leading to the hydrolysis of PIP_2 (5, 6). To gain more insight into the relationship between phospholipase C activation and protein tyrosine kinase activation in human platelets, the effect of genistein on phosphoinositide metabolism was examined. Ca^{2+}-mobilizing stimuli such as thrombin, collagen, and STA_2 stimulate the formation of 1,2-DG and PA in [^{3}H]arachidonic acid-labeled platelets. However, when platelets were pretreated with genistein (10 μg/ml), formation of 1,2-DG and PA induced by collagen (10 μg/ml) or STA_2 (1 μM) was almost completely abolished (Fig. 3). However, genistein did not inhibit thrombin (0.1 unit/ml)-induced phosphoinositide hydrolysis.

Effect of genistein on protein tyrosine phosphorylation. The protein tyrosine phosphorylation was assessed by immunoblotting with antiphosphotyrosine antibodies. Thrombin and STA_2 elevated platelet phosphotyrosine content in several proteins, having molecular weights of about 130,000, 105,000, 95,000, 85,000, 78,000, and 50,000 (Fig. 4). Pretreatment of platelets with genistein resulted in abolition of protein tyrosine phosphorylation induced by 0.3 μM STA_2. In contrast, genistein showed only slight inhibitory effects on thrombin (0.1 unit/ml)-induced protein tyrosine phosphorylation; at concentrations more than 30 μg/ml, it caused weak inhibition of tyrosine phosphorylation of proteins of M_r 105,000, 95,000, 78,000, and 50,000.

Effect of genistein on [^{3}H]U46619 binding. It is well

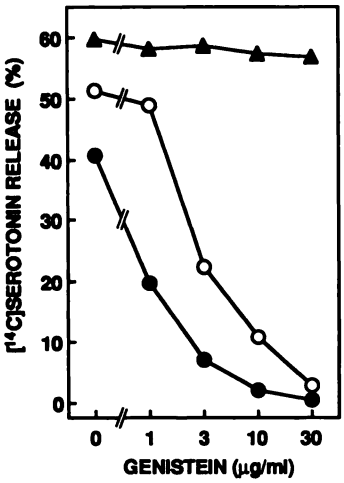

Fig. 2. Effects of genistein on serotonin secretion in human platelets. 5-Hydroxy[^{14}C]tryptamine (serotonin)-labeled platelets were incubated with various concentrations of genistein for 5 min and then stimulated for 1 min with 0.1 μM STA_2 (●), 1.0 μM STA_2 (○), or 0.1 unit/ml thrombin (▲). Each *point* is the mean of triplicate determinations. Two other experiments gave similar results.

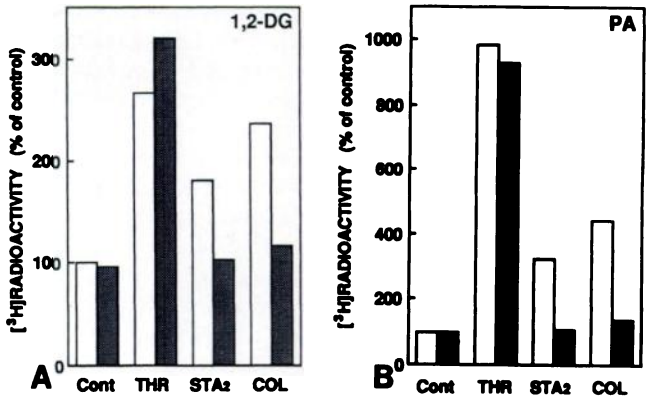

Fig. 3. Effects of genistein on agonist-induced 1,2-DG (A) and PA (B) production. [^{3}H]Arachidonic acid-labeled platelets were incubated in the presence (■) or absence (□) of 10 μg/ml genistein for 5 min, and then stimulated for 3 min with the indicated agent [thrombin (*THR*), 0.1 unit/ml; STA_2, 1.0 μM; collagen (*COL*), 10 μg/ml]. The radioactivity in 1,2-DG (120 dpm) and PA (246 dpm) in unstimulated platelets was designated as 100%, and the remainder of the samples are expressed as a percentage of those values. Other experimental conditions are described in Materials and Methods. The values are means of triplicate determinations. Another experiment gave similar results. *Cont*, control.

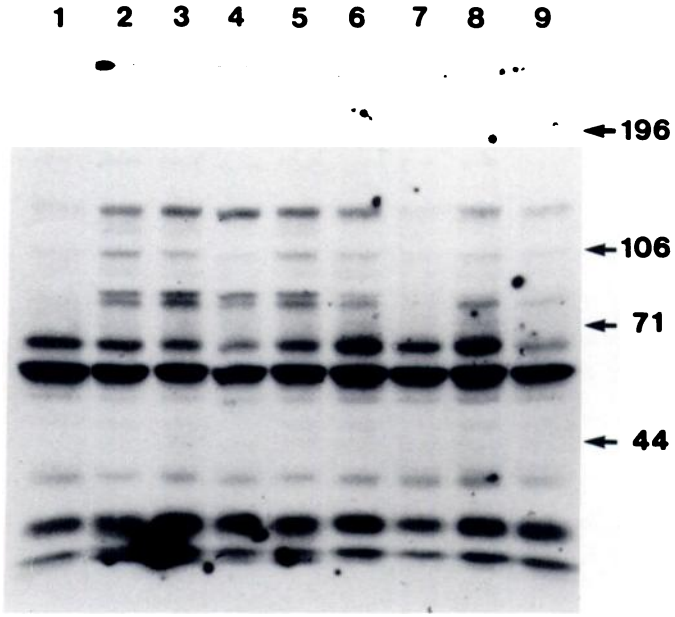

Fig. 4. Effect of genistein and daidzein on agonist-induced protein tyrosine phosphorylation. Platelets were preincubated with genistein or daidzein for 5 min and then stimulated with thrombin (0.1 unit/ml) (*lanes 2–5*) or STA_2 (0.3 μM) (*lanes 6–9*) for 1 min. Tyrosine-phosphorylated proteins were detected by immunoblotting with phosphotyrosine antibodies. *Lane 1*, control; *lanes 2* and *6*, nontreated; *lanes 3* and *7*, genistein, 30 μg/ml; *lane 4*, genistein, 100 μg/ml; *lane 8*, daidzein, 30 μg/ml; *lanes 5* and *9*, daidzein, 100 μg/ml.

demonstrated that collagen-induced platelet activation depends on TXA_2 receptor activation. Therefore, it is likely that the inhibitory effect of genistein on platelet activation induced by collagen or TXA_2 analogs is mediated through blockade of the TXA_2 signal. In the presence of aspirin or TXA_2 antagonists such as ONO-3708, collagen-induced platelet responses were inhibited. The inhibitory profile of genistein for aggregation was similar to that caused by aspirin (data not shown) or ONO-3708 (Fig. 5A). The platelet responses induced by low doses (0.01–0.05 unit/ml) of thrombin were known to be dependent on TXA_2 formation. Genistein, similar to ONO-3708, suppressed platelet aggregation induced by 0.03 unit/ml thrombin (Fig. 5A). The TXA_2 antagonist ONO-3708 reversed aggregation induced by collagen or STA_2. Similarly, genistein disaggregated platelets stimulated with STA_2 or collagen (Fig. 5B). These results strongly suggest that the inhibitory action of genistein is mediated by blockage of the TXA_2 signal. Accordingly, TXA_2 binding analysis was performed using [^{3}H]U46619. It has been shown that [^{3}H]U46619 specifically binds to the platelet TXA_2 receptor (17) and this binding is inhibited by TXA_2 analogs such as STA_2, U46619, and ONO-3708 (data not shown). In the present experiments, Scatchard analysis of [^{3}H]U46619 binding yielded a straight line, indicating a single class of binding sites with a K_d of 116 nM and a B_{max} of about 350 fmol/10^8 platelets. As shown in Fig. 6A, genistein concentration-dependently inhibited [^{3}H]U46619 binding to the TXA_2 receptor. The IC_{50} value was approximately 1.62 μg/ml. The drug competitively inhibited [^{3}H]U46619 binding, with a K_i value of 1.52 μg/ml.

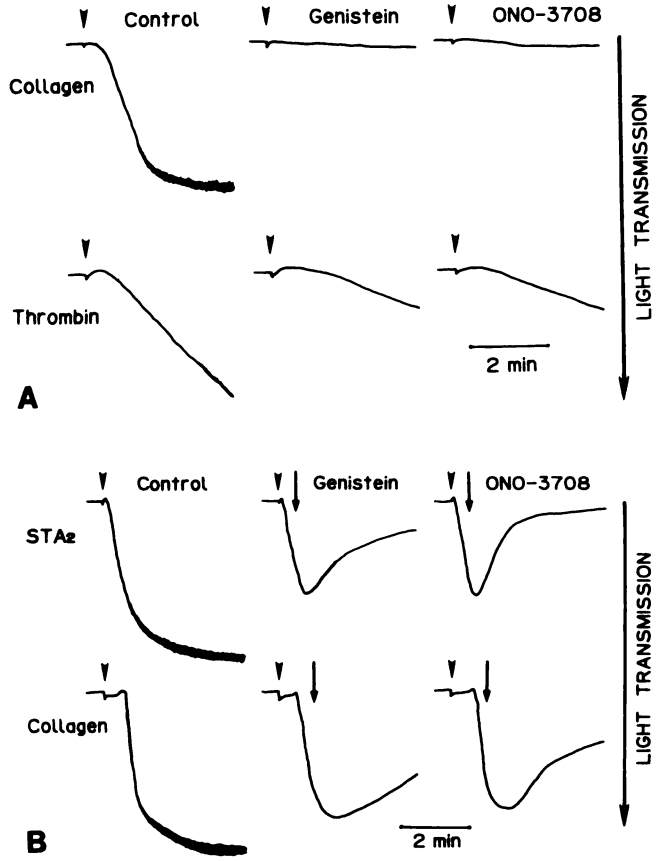

Fig. 5. Effects of genistein and ONO-3708 on human platelet aggregation. A, Washed human platelets were incubated at 37° for 5 min with buffer (*left traces*), genistein (10 μg/ml) (*middle traces*), or ONO-3708 (1 μM) (*right traces*) and then stimulated (*arrowhead*) with 1 μg/ml collagen (*upper traces*) or 0.03 unit/ml thrombin (*lower traces*). B, Washed human platelets were stimulated (*arrowhead*) with 0.1 μM STA_2 (*upper traces*) or 1 μg/ml collagen (*lower traces*). After 30 sec (STA_2) or 60 sec (collagen), 10 μg/ml genistein or 1 μM ONO-3708 was added (*arrow*). The results are shown as typical traces from one experiment, which is representative of three others.

Effects of daidzein on platelet responses. In order to assess whether inhibition of [^{3}H]U46619 binding is specific for genistein, another isoflavone compound, daidzein, was examined for platelet responses. Daidzein has no hydroxyl group at the 5-position of genistein (Fig. 7) and, thus, no inhibitory activity for protein tyrosine kinase (11). Unexpectedly, this drug inhibited collagen- and STA_2-induced platelet aggregation and serotonin secretion (data not shown). Furthermore, daidzein inhibited protein tyrosine phosphorylation induced by STA_2 but not by thrombin (Fig. 4). As shown in Fig. 6B, daidzein also prevented [^{3}H]U46619 binding in a concentration-dependent manner, indicating that the drug inhibited platelet responses by interfering with the TXA_2 signal.

Effects of herbimycin A and ST638 on platelet responses. The effects of other inhibitors of protein tyrosine kinases were also examined. Herbimycin A is a benzoquinonoid ansamycin antibiotic produced by *Streptomyces spp.*, which inhibits growth of Rous sarcoma virus-infected cells with an IC_{50} of 0.45 μg/ml and $p60^{v\text{-}src}$ kinase activity with an IC_{50} of approximately 0.5 μg/ml (19). ST638 is a synthetic 4-hydroxycinnamamide derivative, which inhibits EGF receptor kinase activity with an IC_{50} of 0.44 μM (20). The effects of both drugs on secretory responses are shown in Fig. 8. Neither drug inhibited platelet responses induced by STA_2 or thrombin. The concentrations of both drugs used in the present study were 2- to 5-fold higher than those previously reported to effectively

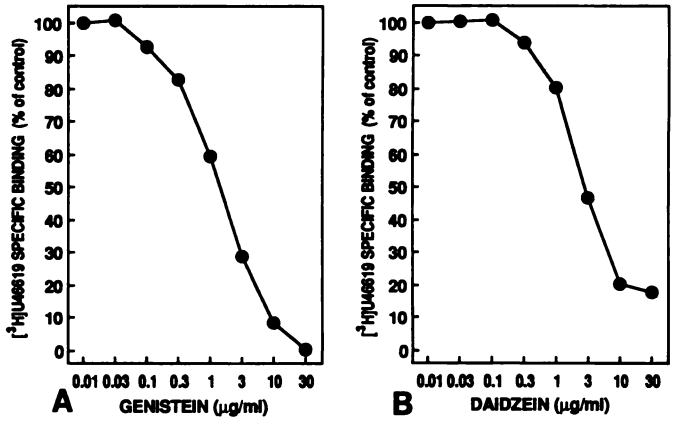

Fig. 6. Inhibition of the specific binding of [^{3}H]U46619 by genistein (A) or daidzein (B). Washed human platelets were incubated with 8 nM [^{3}H]U46619 and various amounts of genistein (A) or daidzein (B), at 37° for 30 min. Specific binding of [^{3}H]U46619 in the absence of the other compounds was designated as 100% and was determined as described in Materials and Methods. Each *point* is the mean of triplicate determinations. Two other experiments gave similar results.

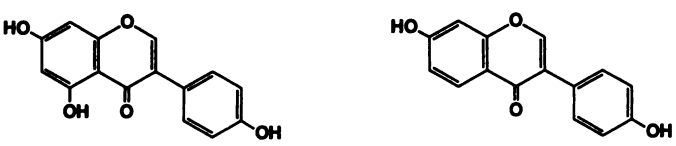

Fig. 7. Structures of genistein and daidzein.

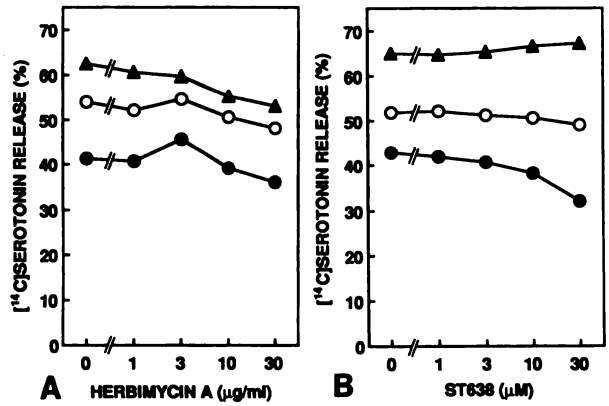

Fig. 8. Effects of herbimycin A (A) and ST638 (B) on serotonin secretion in human platelets. 5-Hydroxy[^{14}C]tryptamine (serotonin)-labeled platelets were incubated with various concentrations of herbimycin A (A) or ST638 (B), for 5 min, and then stimulated for 1 min with 0.1 μM STA_2 (●), 1.0 μM STA_2 (○), or 0.1 unit/ml thrombin (▲). Each *point* is the mean of triplicate determinations. Three other experiments gave similar results.

suppress protein tyrosine kinase activities in other cells. However, herbimycin A (30 μg/ml) and ST638 (30 μM) did not effectively inhibit protein tyrosine phosphorylation induced by thrombin or STA_2.

Discussion

Platelets are known to contain abundant $pp60^{c\text{-}src}$ (2, 3) and other protein tyrosine kinases (21, 22). Because thrombin elevates phosphotyrosine content in platelets (8–10), protein tyrosine phosphorylation is assumed to be involved in platelet signal transduction processes. $pp60^{c\text{-}src}$ is highly enriched in dense bodies (3). Similarly, it has been shown that $pp60^{c\text{-}src}$ is concentrated in chromaffin granules of adrenal medullary cells (23). These observations predict that the oncogene protein would be involved in granule secretion. Ferrell and Martin (24) have reported that protein tyrosine phosphorylation is regulated by platelet glycoprotein IIb-IIIa complex, known as a fibrinogen receptor, suggesting the possibility that protein tyrosine kinase activity is associated with platelet aggregation. In proliferative cells, the association of the protein tyrosine kinase pathway with the phosphoinositide-dependent signal transduction system has been demonstrated (5–7). Recently, thrombin and U46619 were both shown to stimulate the accumulation of phosphoinositides phosphorylated on the 3-position of the inositol ring in human platelets (25, 26). The phosphatidylinositol 3-kinase activity is also thought to be associated with protein tyrosine kinases in growing cells (7). However, the physiological roles of protein tyrosine phosphorylation in platelets, which are terminally differentiated, nonproliferative cells, are still obscure.

Specific inhibitors of protein tyrosine kinase have been used to examine the physiological role of protein tyrosine phosphorylation in growing cells, such as fibroblasts. In the present study, the protein tyrosine kinase inhibitor genistein was applied to human platelets. Two reports have described discrepant results regarding the effect of genistein on PAF-induced rabbit platelet responses (27, 28). Salari *et al.* (27) observed no inhibitory effect on PAF-induced serotonin secretion, whereas Dhar *et al.* (28) reported that genistein blocked PAF-induced rabbit platelet aggregation. The differences are not immediately explained. Genistein inhibited TXA_2-mediated responses in human platelets. Moreover, another isoflavone compound, which lacks inhibitory activity for protein tyrosine kinase, also suppressed TXA_2-stimulated platelet responses. These isoflavone compounds blocked TXA_2 binding to platelets (Fig. 6). Genistein competitively inhibited [^{3}H]U46619 binding to the platelets, with a K_i value of 1.52 μg/ml (about 5.6 μM), which is far higher than those of well known TXA_2 antagonists such as SQ29548 (7.6 nM) and ONO-3708 (37 nM) (17). However, the IC_{50} for [^{3}H]U46619 binding (1.62 μg/ml) is very close to that for EGF receptor kinase (0.75 μg/ml) (11). The concentration-dependent inhibitory profile of TXA_2 binding correlated well with that of STA_2-induced serotonin secretion. [^{3}H]U46619 binding was almost completely inhibited at 10 μg/ml levels of the drug (Fig. 6A). At the same concentration, STA_2 (0.1 μM)-induced serotonin secretion was abolished (Fig. 2). Gaudette and Holub (29) reported inhibitory effects of genistein on U46619-induced phosphoinositide phosphorylation. Although the authors discuss the possible regulation of phosphatidylinositol kinase(s) by protein tyrosine kinase, this inhibitory action could also be due to the attenuation of the U46619 signal by prevention of drug binding to the receptor. The structures of genistein and daidzein, which are illustrated in Fig. 7, are quite dissimilar to that of TXA_2. However, compounds such as BM-13177 (17) and trimethoquinol (30), which have different structures from prostaglandins, are known to act as TXA_2 antagonists. The primary aggregation induced by adrenaline and ADP was not prevented by genistein, suggesting that the drug did not interfere with ADP and adrenaline signals.

Genistein did not inhibit thrombin-induced platelet responses, although thrombin treatment of platelets led to the protein tyrosine phosphorylation of several proteins. After a 3-hr incubation of platelets with the drug, thrombin-induced platelet responses, including protein tyrosine phosphorylation, were hardly affected (data not shown). Thus, genistein does not appear to be a particularly good inhibitor of protein tyrosine kinase in intact platelets. Inhibitors of protein tyrosine kinases exhibit selectivity toward different types of protein tyrosine kinase. Other protein tyrosine kinase inhibitors, herbimycin A and ST638, did not show any significant inhibitory action on thrombin- or TXA_2-induced human platelet responses (Fig. 8). After a 3-hr incubation, neither drug showed inhibition. Neither drug inhibited protein tyrosine phosphorylation (data not shown), indicating that these drugs are not suitable protein kinase inhibitors in intact platelets. Further studies are necessary to clarify the involvement of protein tyrosine kinases in the platelet signal transduction system.

Acknowledgments

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