Effects of Tyrosine Kinase Inhibitors on Antigen Challenge of Guinea Pig Lung In Vitro

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ABSTRACT

The present study was conducted to examine the effects of two protein tyrosine kinase inhibitors, genistein and tyrphostin 47, on an in vitro model of allergic asthma. Guinea pigs were sensitized with purified IgG raised against ovalbumin (OA). Isolated sensitized bronchial rings contracted in response to OA in a concentration-dependent manner, maximum contraction being achieved at 1 μg/ml. Genistein and tyrphostin 47 concentration-dependently (10–100 μM) inhibited OA-induced anaphylactic contraction of the bronchi, as well as release of histamine and peptidoleukotrienes from chopped lung preparations. Genistein, but not tyrphostin 47, significantly suppressed bronchial contraction to leukotriene D4 at 50 μM and to histamine at 100 μM. Daidzein, an inactive congener of genistein, did not alter OA-induced anaphylactic contraction. However, it slightly reduced bronchial contraction to leukotriene D4 and the OA-stimulated release of peptidoleukotrienes. The inhibitory effects were significantly weaker than those of genistein. Taken together, our results show that tyrphostin 47 inhibited anaphylactic contraction mainly by preventing mast cell degranulation, whereas genistein exerted inhibitory effects partly by blocking mast cell degranulation and partly by attenuating leukotriene D4-induced bronchial contraction. These findings suggest that protein tyrosine kinase inhibitors have a therapeutic potential as mast cell stabilizers in the treatment of allergic diseases such as bronchial asthma.

Antigen-stimulated mast cell degranulation is known to be associated with increased intracellular Ca++ concentration and protein kinase C activity (Beaven and Metzger, 1993; Ozawa et al., 1993). Recently, cumulating evidence obtained from rat basophilic mast cell line (RBL-2H3) and bone marrow-derived mast cells showed that activation of non-transmembrane PTKs is the earliest detectable signaling response to FcεRI cross-linking. This is followed by downstream signaling events such as activation of PLCγ (Li et al., 1992; Jouvin et al., 1994) and mitogen-activated protein kinase (Fukamachi et al., 1993), increase in inositol 1,4,5-trisphosphate and intracellular Ca++ levels and enhanced protein kinase C activity, and it eventually leads to mast cell degranulation (Beaven and Metzger, 1993; Scarenberg and Kinet, 1994). Specific tyrosine kinases such as src-related kinase Lyn (Eiseman and Bolen, 1992), 72-kDa Syk (Hutchcroft et al., 1992; Benhamou et al., 1993), 94-kDa Fer (Penhallow et al., 1995) and 77-kDa Btk (Kawakami et al., 1994) have been shown to be activated rapidly after FcεRI aggregation. Inhibitors of PTK have been shown to block antigen-induced activation of PTK, related downstream signaling events (e.g., inositol 1,4,5-trisphosphate production) and histamine release from mast cells (Kawakami et al., 1992; Lavens et al., 1992; Oliver et al., 1994). Because mast cell degranulation is the hallmark of immediate-type hypersensitivity reaction, which is also the major mechanism for a variety of allergic diseases such as bronchial asthma, it is logical to examine the effects of PTK inhibitors on an in vitro model of allergic asthma.

The Schultz-Dale reaction (Schultz, 1910; Dale, 1913; Chand and Eyre, 1978) has been used extensively to study anaphylactic contraction of airway tissue preparations such as trachea, bronchi and lung parenchymal strips. Among a wide array of mast cell-derived inflammatory mediators, such as thromboxane A2 and platelet-activating factor, peptidoleukotrienes and histamine have been shown to be the major mediators responsible for the anaphylactic contraction of the airways (Ro et al., 1991; Bjorck and Dahlen, 1993; Jonsson and Dahlen, 1994). A combination of histamine (H1) receptor antagonist and peptidoleukotriene receptor antagonist has been shown to block substantially the anaphylactic contraction of airway tissue preparations (e.g., bronchi and lung parenchymal strips) from both human and guinea pig (Regal, 1985; Bjorck and Dahlen, 1993; Jonsson and Dahlen, 1994). In guinea pigs, both IgE and IgG are able to sensitize mast cells to specific antigen (Regal, 1985; Undem et al., 1990).

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ABBREVIATIONS: OA, ovalbumin; LTD4, leukotriene D4; HNMT, histamine N-methyltransferase; PTK, protein tyrosine kinase; FcεRI, high affinity IgE-binding Fc receptor; FcγR, IgG-binding Fc receptor; [3H]-methylhistamine; PLCγ, phospholipase Cγ.
cell degranulation and partly by attenuating LTD4-induced contraction. Tyrphostin 47 acted on mast cell degranulation. It has been shown that IgG-sensitized guinea pig airway tissues released more histamine and peptide leukotrienes (Undem et al., 1985; Ro et al., 1991) and that their anaphylactic contractions were more sensitive to inhibition by combined H1-receptor and peptide leukotriene receptor antagonism, results that closely resemble the IgE-dependent anaphylactic responses in human (Regal, 1985; Bjorek and Dahlen, 1993). FcγR (e.g., FcγRII) and FcεRI are structurally and functionally related, and both belong to a family of multuisubunit antigen receptors (Alber et al., 1992; Bolen, 1995). It has been shown that engagement of these cell surface receptors activates PTKs as the initial events for successful signal propagation (Bolen, 1995).

In this study, we passively sensitized guinea pigs with IgG raised against OA and studied the effects of two PTK inhibitors, genistein, and tyrphostin 47 (Gazit et al., 1989; Levitzki and Gazit, 1995), on antigen-induced anaphylactic contraction of the bronchi and release of histamine and peptide leukotrienes from chopped lung preparations. Our findings show that genistein and tyrphostin 47 substantially inhibited both antigen-induced release of mediators and anaphylactic contraction. Tyrphostin 47 acted mainly by preventing mast cell degranulation, whereas genistein exerted inhibitory effects partly by blocking mast cell degranulation and partly by attenuating LTD4-induced bronchial contraction. Taken together, these data suggest that PTK inhibitors have a therapeutic potential as mast cell stabilizers in the treatment of allergic diseases such as bronchial asthma.

Materials and Methods

Drugs. The following drugs and chemicals were used in this study: OA (grade V), histamine dihydrochloride, indomethacin, tyrphostin 47 (RG50864), L-cysteine, bovine serum albumin (Sigma Chemical Co., St. Louis, MO), toluene, isoamyl alcohol, boric acid, potassium phosphate, dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany), rabbit IgG fraction to chicken egg albumin (OA) (Organon Teknica Corp., Durham, NC), genistein and daidzein (Research Biochemicals International, Natick, MA), LTD4 (Cayman Chemical Co., Ann Arbor, MI), tritiated S-adenosyl-L-[methyl-3H]methionine (60–85 Ci/mmol), leukotriene C4/D4/E4 radioimmunoassay kit and biodegradable liquid scintillant (Amersham Life Science, Buckinghamshire, U.K.) and HNMT (New England Nuclear, Boston, MA). Rabbit anti-OA IgG antibody was stored in sterile H2O, rabbit IgG fraction to chicken egg albumin (OA) (Organon Teknica Corp., Durham, NC) and genistein (Research Biochemicals International, Natick, MA), LTD4 (Cayman Chemical Co., Ann Arbor, MI), tritiated S-adenosyl-l-[methyl-3H]methionine (60–85 Ci/mmol), leukotriene C4/D4/E4 radioimmunoassay kit and biodegradable liquid scintillant (Amersham Life Science, Buckinghamshire, U.K.) and HNMT (New England Nuclear, Boston, MA). Rabbit anti-OA IgG antibody was stored in sterile H2O, rabbit IgG fraction to chicken egg albumin (OA) (Organon Teknica Corp., Durham, NC), genistein and tyrphostin 47 (47) and daidzein in DMSO at 4°C for 3 mm before use.

Preparation of bronchial rings. Male Hartley guinea pigs (Interauna, U.K. Ltd. England) weighing 350 to 450 g were sacrificed by CO2 asphyxiation and subsequent decapitation. After thoracotomy, heart and lung were excised en bloc and perfused with 50 ml of Krebs-bicarbonate solution via the pulmonary artery. Lung lobes were isolated for studies on the release of histamine and peptide leukotrienes (see below). Bronchial rings (~3 mm in length) were obtained from the hilar bronchi and cleaned of any parenchyma. Ring preparations were then suspended isometrically under an optimum resting load of 2 g in organ baths containing 10 ml of Krebs-bicarbonate solution aerated with 95% O2 and 5% CO2 at 37°C and of the following composition (mM): NaCl, 118.2; KCl, 4.6; NaHCO3, 24.8; CaCl2, 2.0; H2O, 2.5; KH2PO4, 1.2; MgSO4, 7.0; H2O, 1.2 and dextrose, 10.0. Contractile responses were monitored using force-displacement transducers (Grass FT-03) coupled to a MacLab/8 data-recording system (ADInstruments, Castle Hill, Australia). A 90-min equilibration period was allowed before any experimentation was begun, and during this time the bath fluid was changed every 10 min.

Contractile studies. After equilibration, bronchial rings were contracted with 60 mM KCl. The contraction was defined as the maximum tissue response, and all subsequent contractions were compared to it. For antigen challenge studies, ring preparations were preincubated with indomethacin (4 μM) for 30 min before addition of OA. Indomethacin has been shown to reduce the production of relaxant prostanoids (e.g., PGE2 and PGF2α) capable of modulating the anaphylactic contraction (Abela and Daniel, 1994; Bertrand et al., 1991; Ro et al., 1991). To determine maximum antigen-induced contraction, bronchial rings were exposed to increasing concentrations of OA (0.001–10 μg/ml). To evaluate the role of PTK in mediating bronchial smooth muscle anaphylactic contraction, PTK inhibitors such as genistein, tyrphostin 47 and daidzein, a structural analog of genistein devoid of tyrosine kinase inhibitory activity (Akiyama et al., 1987), were preincubated with bronchial rings 30 min before addition of OA. The effects of PTK inhibitors were compared to those of their corresponding control ring preparations in the absence of inhibitor. To confirm that the inhibitory effects of PTK inhibitors on anaphylactic contraction are mediated by mast cell stabilization, we examined the effects of genistein and tyrphostin 47 on histamine- or LTD4-induced bronchial contraction. In the LTD4-induced bronchial ring contraction study, 4 μM indomethacin and 5 mM l-cysteine were preincubated with the tissue for 30 min. It has been reported that very minute amounts of relaxant prostanoids (e.g., PGE2 and PGF2α) were able to mask LTD4-induced canine bronchial contraction and that the addition of indomethacin restored the LTD4 effect (Abela and Daniel, 1994). L-cysteine is an inhibitor of an aminopeptidase that converts LTD4 to less potent LTE4. Adding L-cysteine has been shown to enhance the smooth muscle contractile response to LTD4 (Abela and Daniel, 1994).

Release of mediators from chopped lung preparations. Lung lobes obtained from sensitized guinea pigs were cut into approximately 1-mm3 pieces using a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY). Fragmented lung preparations were washed thoroughly with oxygenated Krebs-NaHCO3 buffer before incubation. Duplicate aliquots of 200-μg lung fragments were weighed and placed in plastic scintillation vials containing 2 ml of oxygenated Krebs solution in the presence of 4 μM indomethacin (for histamine release) plus 5 mM cysteine (for release of peptide leukotrienes). Lung samples were then incubated in a shaker bath at 37°C for 45 min before they were challenged with OA for 5 min (histamine release) or 10 min (release of peptide leukotrienes) (Wong et al., 1992b). To determine the mast cell-stabilizing effects of tyrosine kinase inhibitors, they were preincubated with the lung preparation for 30 min before immunologic stimulation. Diffusates were then collected and stored at −70°C until assay.

Histamine radioenzymatic assay. Histamine release from lung samples in response to OA was determined via a radioenzymatic assay as described by Henry et al. (1991), utilizing highly purified HNMT. Briefly, a total incubation volume of 60 μl was prepared by sequential addition of 10 μl of biological samples (or H2O for the blank), 25 μl of H2O2 (or H2O containing 500 pg of histamine as internal standard) and 25 μl of reaction reagent in 12 × 75-mm polypropylene culture tubes. Reaction reagent contained 21 μl of 0.4 M potassium phosphate/0.1% bovine serum albumin, pH 7.8, 2 μl of HNMT, and 2 μl of tritiated S-adenosylmethionine (80 Ci/mmole). After a 1-hr incubation in a shaker bath at 2°C, the enzymatic reaction was terminated by the addition of 75 μl of 2.5 M potassium...
borate, pH 11; 4 ml of toluene-isooamyl alcohol (3:1, v/v) was then added to each tube. After centrifugation for 3 min, 3.8 ml of the organic phase, which contained tritiated N-α-methylhistamine ([3H]-α-MHm) formed by the HNMT reaction, was transferred to another set of tubes containing 500 μl of 0.5 M HCl for back extraction of the [3H]-α-MHm into the aqueous phase. Tubes were centrifuged for 3 min, and the organic phase was aspirated and discarded. The aqueous phase was mixed with 1.25 ml of toluene-isooamyl alcohol. After centrifugation and removal of the organic phase, 300 μl of the acidified aqueous phase was transferred to scintillation vials containing 8 ml of biodegradable counting scintillant. Radioactivity was quantitated by liquid scintillation spectrometry (Beckman LS 3801, Beckman Instruments, Inc., Fullerton, CA).

Leukotrienes radioimmunoassay. The release of peptidoleukotrienes from chopped lung preparations in response to OA was quantitated by radioimmunoassay (Amersham Life Science, Buckinghamshire, U.K.). Briefly, total incubation volumes of 400 μl were prepared by sequential addition of 100 μl of biological samples or LTC4 standard, 100 μl of [3H]-LTC4, 100 μl of peptidoleukotriene-specific antiserum (cross-reactivity for LTC4/D4/E4 = 100%/100%/41%), and 100 μl of assay buffer (pH 7.4) in polypropylene tubes. Antigen-antibody competition reaction was allowed to take place overnight at 2°C to 8°C. Dextran-coated charcoal suspension (250 μl) was then added to each reaction tube to adsorb any unbound leukotrienes. After centrifugation, the supernatant was transferred to scintillation vials containing 10 ml of scintillant. Radioactivity was measured using liquid scintillation spectrometry. Samples were assayed in duplicate.

Data analysis. All data are presented as mean ± S.E.M. Statistical differences in contractile responses to OA challenge, histamine or LTD4, and in the release of mediators in response to OA, in the presence and absence of inhibitors were analyzed using ANOVA followed by Student-Newman-Keuls test (Armitage and Berry, 1987). The critical level for significance was set at P < .05.

Results

Antigen challenge. Sensitized guinea pig bronchial rings contracted in response to 60 mM KCl with an active force of contraction that amounted to 1.45 ± 0.10 g (n = 19). It also produced graded contractile responses to increasing concentrations of OA (fig. 1A). The sensitized bronchial rings did not contract to irrelevant protein such as bovine serum albumin. The threshold concentration of OA to induce anaphylactic contraction was 0.01 μg/ml, and maximum response was achieved at 1 μg/ml. The concentration that caused 50% maximum contraction was 0.05 μg/ml (fig. 1B). For all subsequent antigen-challenge studies, OA at a concentration of 1 μg/ml was used to induce maximum anaphylactic bronchial smooth muscle contraction (1.83 ± 0.10 g, n = 19).

Effects of PTK inhibitors on Schultz-Dale reaction. To determine the effects of PTK inhibitors on the Schultz-Dale reaction, the inhibitors were preincubated with the ring preparations for 30 min before OA challenge. Genistein concentration-dependently inhibited OA-induced bronchial contraction by 15%, 60%, 73% and 99% at 10 μM, 30 μM, 50 μM and 100 μM, respectively (fig. 2A). In contrast, 50 μM daidzein, a structural analog of genistein devoid of tyrosine kinase inhibition activity, did not affect OA-induced bronchial contraction. On the other hand, tyrphostin 47 significantly inhibited anaphylactic contraction by 25%, 31%, 73% and 77% at 10 μM, 30 μM, 50 μM and 100 μM, respectively (fig. 2B).

Effects of PTK inhibitors on mediator-induced bronchial contraction. To determine whether the inhibition of anaphylactic contraction is mediated by blocking the release of mast cell-derived mediators or by attenuating bronchial smooth muscle contraction, we evaluated the PTK inhibitors in histamine- or LTD4-induced bronchial ring contraction. At 50 μM, a concentration that significantly blocked the OA-induced bronchial contraction, neither genistein nor tyrphostin 47 attenuated bronchial contraction (1.45 ± 0.07 g, n = 26) induced by 30 μM histamine (fig. 3A). At 100 μM, genistein but not tyrphostin 47 substantially suppressed histamine-induced bronchial contraction by 88%. Diadzein (100 μM) did not exhibit any inhibitory effect on histamine-induced bronchial contraction. Bronchial contraction induced by 0.1 μM LTD4 (1.45 ± 0.06 g, n = 14) was significantly reduced by genistein (75%), but not by tyrphostin 47, at a concentration of 50 μM. Daidzein (50 μM) also inhibited LTD4-induced contractile response by 44%. Nevertheless, the inhibitory effect of daidzein was significantly (P < .05) less than that of genistein (fig. 3B).
Effects of PTK inhibitors on the release of mediators. Chopped lung preparations released low levels of histamine (75.5 ± 21.0 ng/g tissue, n = 13) and peptidoleukotrienes (1.2 ± 0.1 ng/g tissue, n = 8) spontaneously. Upon 1 µg/ml OA challenge, the release of histamine and that of peptidoleukotrienes from lung fragments were significantly increased by 24-fold (1809.0 ± 135.2 ng/g tissue, n = 13) and 60-fold (71.6 ± 5.7 ng/g tissue, n = 8), respectively. Genistein concentration-dependently inhibited antigen-induced histamine release by 32%, 54% and 67% at 10 µM, 50 µM and 100 µM, respectively (fig. 4A). Likewise, it substantially reduced OA-induced release of peptidoleukotrienes by 19%, 58% and 74% at 10 µM, 50 µM and 100 µM, respectively (fig. 4B). In contrast, daidzein (50 µM), failed to block histamine release but significantly reduced the release of peptidoleukotrienes from lung fragments by 29%. Nevertheless, the inhibitory effect of daidzein on the release of peptidoleukotrienes was significantly (P < .05) weaker than that of genistein. On the other hand, tyrphostin 47 at concentrations of 10 µM, 50 µM and 100 µM significantly reduced histamine release from lung fragments by 22%, 41% and 48%, respectively, and markedly blocked the release of peptidoleukotrienes by 40%, 92% and 98%, respectively (fig. 4). The inhibitory effect of tyrphostin 47 on the release of leukotrienes was substantially more pronounced than that of genistein.
inhibition for epidermal growth factor receptor kinase 800 times more potently than for insulin receptor kinase (Levitzki and Gazit, 1995). Their relative potencies against non-transmembrane PTKs (e.g., Jak, Fer, Syk, Btk, and Lyn) remain to be determined.

Bronchial rings from guinea pigs passively sensitized with purified IgG raised against OA contracted in response to OA, but not to bovine serum albumin, in a concentration-dependent manner (fig. 1). This observation is consistent with our previous study using guinea pig lung parenchymal strips (Wong et al., 1992a) and with other reports using guinea pig trachea (Undem et al., 1985; Bertrand et al., 1991; Ro et al., 1991), which showed that IgG is able to sensitize guinea pig mast cells to specific antigen. Both IgG-binding FcγRI (e.g., FcγRIII) and IgE-binding FcεRI are expressed on the surface of human and murine mast cells (Ravetch and Kinet, 1991; Alber et al., 1992). IgG-mediated aggregation of FcγRIII has been shown to stimulate the release of arachidonic acid metabolites (Alber et al., 1992) and serotonin (Daeron et al., 1992) from murine mast cells. In an effect analogous to that of FcεRI, cross-linking of two or more FcγRs triggers non-transmembrane PTK activities as the initial signaling events, followed by increases in intracellular Ca²⁺ level and protein kinase C activity, which in turn leads to exocytosis of mast cell-derived inflammatory mediators (Alber et al., 1992; Beaven and Metzger, 1993).

OA-induced anaphylactic contraction of the bronchi was significantly inhibited by either genistein or tyrphostin 47 in a concentration-dependent manner (fig. 2). At 50 μM, both genistein and tyrphostin 47 markedly suppressed bronchial anaphylactic contraction by at least 70%. In contrast, 50 μM daidzein, an analog of genistein that has no inhibitory activity for PTK (Akiyama et al., 1987), failed to alter the anaphylactic contraction. On the other hand, the OA-induced release of histamine and peptidoleukotrienes from chopped lung preparations was also concentration-dependently reduced by genistein and tyrphostin 47 (fig. 4). Lavens et al. (1992) previously showed that genistein inhibited anti-IgE-induced histamine release from human lung mast cells. These findings suggest that PTK is involved in the Schultz-Dale reaction and that the inhibitors of PTK interrupted the early signaling pathway of FcγR cross-linking in the mast cells (Alber et al., 1992; Bolen, 1995) and, therefore, attenuated the anaphylactic contraction by preventing the release of mast cell-derived inflammatory mediators such as histamine and peptidoleukotrienes.

In order to ascribe the inhibitory effects of genistein and tyrphostin 47 on anaphylactic contraction solely to mast cell stabilization, we must first determine whether the PTK inhibitors themselves have any effects on the bronchial smooth muscle contraction. Our current understanding of the biological activities of PTK has been derived mainly from studies of growth factor-associated responses such as mitogenesis, differentiation and proliferation of immune cells (Bolen, 1995; Levitzki and Gazit, 1995). Lately, evidence has been accumulating that protein tyrosine phosphorylation also participates in the regulation of smooth muscle contraction (Di Salvo et al., 1994; Hollenberg, 1994; Semenchuk and Di Salvo, 1995; Jin et al., 1996). In addition to playing a central role in the EGF-induced gastric smooth muscle contraction (Hollenberg, 1994), PTK mediates vascular smooth muscle contraction induced by serotonin or phenylephrine (Semenchuk and Di Salvo, 1995; Watts et al., 1996) and gastric longitudinal smooth muscle contraction induced by angiotensin-II (Yang

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**Discussion**

Genistein and tyrphostin 47 are structurally and functionally unrelated inhibitors of PTK (Levitzki and Gazit, 1995). Genistein is an isoflavone compound that inhibits PTK activity of the epidermal growth factor receptor and pp60c-src via competitive inhibition at the ATP-binding domain of the kinases (Akiyama et al., 1987; Di Salvo et al., 1993). Tyrphostin 47 (RG50864) is a derivative of the dihydroxybenzylidene malononitrile class of PTK inhibitors that acts by competitive inhibition at the substrate site of the kinase (Gazit et al., 1989). Genistein is a broad-spectrum PTK inhibitor; the ATP binding site that it inhibits has been shown to be highly conserved among PTKs. Tyrphostin 47 exhibits selective inhibition for epidermal growth factor receptor kinase 800 times more potently than for insulin receptor kinase (Levitzki and Gazit, 1995).
et al., 1993). These findings indicate that PTK is involved in the signal transduction of neurotransmitters known to act through G protein-coupled receptors. Inhibitors of PTK such as genistein and tyrphostins have been shown to inhibit smooth muscle contraction to carbachol, norepinephrine, phenylephrine, serotonin and angiotensin-II (Di Salvo et al., 1993; Yang et al., 1993; Abebe and Agrawal, 1995; Watts et al., 1996).

At a concentration (50 μM) that significantly inhibited OA-induced anaphylactic contraction and mediator release, neither genistein nor tyrphostin 47 altered histamine-induced bronchial contraction. However, as the concentration was increased to 100 μM, genistein, but not tyrphostin 47, abolished bronchial contraction induced by histamine. As expected, 100 μM daidzein had no effect on histamine-mediated contraction (fig. 3A). The inhibitory effect of genistein might be related to the substantial inactivation of certain PTKs that participate in the signal transduction of histamine-induced bronchial contraction. It has been shown in human umbilical vein endothelial cells that histamine induced a delayed tyrosine phosphorylation of the 42/44-kDa mitogen-activated protein kinase, which suggests that PTK is a mediator in the histamine signal transduction cascade (Fleming et al., 1995). However, it remains to be determined whether tyrosine phosphorylation of the same or other substrates occurs in histamine-treated bronchial smooth muscle cells. Alternatively, genistein at a concentration of 100 μM might have exhibited some nonspecific activities against histamine-induced bronchial contraction. Wijetunge et al. (1992) showed that 100 μM genistein, but not daidzein, inhibited voltage-operated calcium channel currents in vascular smooth muscle. However, we still do not know whether the inhibition of calcium channel currents is a direct blockade by genistein or a result of PTK inactivation.

On the other hand, LTD4-induced bronchial smooth muscle contraction was significantly inhibited by genistein, but not by tyrphostin 47, at 50 μM concentration. Daidzein (50 μM) also attenuated the LTD4-mediated contraction, but its inhibitory effect was significantly weaker than that of genistein (fig. 3B). The effect of genistein on LTD4-induced contraction is unlikely to be associated with nonspecific activity on other serine/threonine kinases. It has been shown that genistein scarcely inhibited cAMP-dependent protein kinase, protein kinase C, phosphorylase kinase and phosphorylase b, and even at concentrations above 300 μM (Akiyama et al., 1987). Another possible explanation for genistein’s inhibitory effect is blockade of Ca++ channel currents in the bronchial smooth muscle. This does not appear to be the case, however, because 50 μM genistein failed to block the histamine-induced bronchial contraction. Thus it is likely that genistein attenuated LTD4-induced contraction by mitigating the PTK activities stimulated by LTD4 receptor activation. It has been demonstrated in human epithelial cells that LTD4 induced tyrosine phosphorylation of PLCγ and of two other associated protein substrates (Gronroos et al., 1995). Activation of PLCγ produces inositol 1,4,5-trisphosphate and diacylglycerol, resulting in increased intracellular Ca++ concentration and protein kinase C activity, which ultimately regulate smooth muscle contractility (Hollenberg, 1994).

The inhibitory effects of daidzein on LTD4-induced bronchial contraction were unexpected, given the reported inactivity of this compound on EGF receptor kinase and pp60src (Akiyama et al., 1987). In addition, 50 μM daidzein reduced the release of peptidoleukotrienes from lung fragments. Nevertheless, the inhibitory effects of daidzein are significantly weaker than those of genistein (figs. 3 and 4). Recently, daidzein has been shown to attenuate serotonin- , norepinephrine- or KCl-induced vascular smooth muscle contraction (Toma et al., 1995; Watts et al., 1996). These recent findings, together with our results, suggest that daidzein might possess some activity against PTK (Toma et al., 1995; Watts et al., 1996). Alternatively, the inhibitory effects of both genistein and daidzein on LTD4-induced bronchial contraction and peptidoleukotrienes release from lung fragments might be due to certain unidentified nonspecific effects of the drugs.

In summary, genistein and tyrphostin 47 substantially inhibited the Schultz-Dale reaction in the guinea pig airways. Genistein exhibited its modulatory effects partly by mast cell stabilization and partly by reduction of smooth muscle contraction in response to LTD4. Tyrphostin 47 acted primarily as a mast cell stabilizer without having any significant effect on smooth muscle contraction. Because mast cells play a pivotal role in initiating allergic disorders such as bronchial asthma (Wasserman, 1994), our findings suggest that PTK inhibitors may have therapeutic potential as mast cell stabilizers in the treatment of asthma.

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