Mechanism of Inhibitory Action of Tranilast on the Release of Slow Reacting Substance of Anaphylaxis (SRS-A) In Vitro: Effect of Tranilast on the Release of Arachidonic Acid and Its Metabolites

Hidetada KOMATSU, Masami KOJIMA, Naoyuki TSUTSUMI, Shuichiro HAMANO, Hiroshi KUSAMA, Arao UJIIE, Shigeru IKEDA and Masayuki NAKAZAWA
Central Research Laboratories, Kissei Pharmaceutical Co., Ltd., Matsumoto 399, Japan
Accepted September 30, 1987

Abstract—We investigated the mechanism of inhibitory action of tranilast, one of the anti-allergic drugs, on the release of slow reacting substance of anaphylaxis (SRS-A). Ionophore A23187 (0.5 or 0.2 µg/ml)-induced SRS-A release from rat peritoneal exudate cells (PEC) or human leucocytes was inhibited by tranilast (10^{-5} - 10^{-3} M). The IC50 (the concentration which gives 50% inhibition) of tranilast on these reactions was approx. 10^{-4} M. Prostaglandin (PG)E_2 release from sensitized purified rat mast cells (PMC) by a specific antigen (DNP-Ascaris) was markedly suppressed by tranilast (10^{-3} M). Similarly, ionophore A23187-induced PGE_2 and 6-keto-PGF_1α releases from rat PEC were inhibited by tranilast (10^{-5} - 10^{-3} M). DNP-Ascaris antigen-induced 3H-arachidonic acid (AA), 3H-PGE_2, 3H-PGF_2α and 3H-PGD_2 releases from rat PMC were markedly suppressed by tranilast (10^{-6} - 10^{-3} M), DSCG (10^{-5} - 10^{-4} M) and mepacrine (10^{-3} M). The activity of AA-converting enzymes such as 5-lipoxygenase, cyclooxygenase, PG_12 synthetase, and glutathione-S-transferase was hardly influenced by tranilast (10^{-5} - 10^{-3} M). From these results, we suggest that the mechanism of the inhibitory action of tranilast on the release of SRS-A is related to the processes prior to dissociation of AA from the membrane phospholipids.

Histamine, slow reacting substance of anaphylaxis (SRS-A), prostaglandins (PGs) and chemotactic factors are released in immediate hypersensitivity reactions. Mast cells or basophils play an important role because of their ability to release histamine during antigen-antibody reactions (1), but SRS-A and PGs, oxidative metabolites of arachidonic acid (AA), are generated or released from other tissues and cells rather than from mast cells, by non-immunological stimulations (2-4). N-(3, 4-dimethoxycinnamoyl) antranilic acid (tranilast), one of the anti-allergic drugs, has been reported to inhibit the release of histamine and SRS-A from rat peritoneal exudate cells (PEC) and guinea-pig lungs in antigen-antibody reactions (5-11). However, the mechanism of the inhibitory action of tranilast on the release of chemical mediators in immediate hypersensitivity reactions remains obscure.

In this study, to elucidate mechanisms of the inhibitory action of tranilast on the release of SRS-A, we investigated the effect of tranilast on the release of AA and its metabolites in rat PEC, rat purified mast cells (PMC) and human leucocytes and determined its effects on the activities of AA-converting enzymes.

Materials and Methods
Preparation of rat PMC and PEC suspensions
a) Rat PMC: Murine hybridoma secreting monoclonal IgE antibodies with anti-2, 4-
dinitrophenyl (DNP) specificity was obtained by the fusion of X63Ag8 tumor cells and spleen cells from DNP-Ascaris-hyperimmunized BALB/c mice. The supernatant of cultured murine hybridoma gives a threshold reaction of passive cutaneous anaphylaxis (PCA) at a dilution of 1/2000 in rats. The supernatant was injected into the pleural and peritoneal cavities of male Wistar rats. After 16 hr, animals were exsanguinated by decapitation, and the exudate cells were harvested from the pleural and peritoneal cavities with medium of the following composition: 150 mM NaCl, 3.7 mM KCl, 3.0 mM Na2HPO4, 12H2O, 3.5 mM KH2PO4, 0.9 mM CaCl2·2H2O, 5.6 mM glucose, 0.1% bovine serum albumin (BSA) and 5 units/ml heparin, pH 6.9. The cell suspension was centrifuged at 35×g for 10 min at 4°C and layered over 5.0 ml of a solution of 38% BSA as described by Sullivan et al. (12). The cells were allowed to settle for 20 min and centrifuged at 460×g at room temp. for 20 min. Consequently, a mast cell population with a purity of approx. 90% was obtained from the BSA layer.

b) Rat PEC: Male Wistar rats were killed by a blow on the head and exsanguination by decapitation. Rat PEC suspension was prepared as described by Nakazawa et al. (8) using a phosphate-buffered salt solution (PBS) containing 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2·6H2O, 5.6 mM glucose, 5 units/ml heparin and 5% (v/v) phosphate (0.1 M, 7.2).

c) Human leucocytes: Ten ml of citrated blood from healthy humans were mixed with 2.0 ml of 4% dextran (M.W.=180000, Nakarai chemicals Kyoto, Japan) dissolved in 0.9% sodium chloride. The mixture was allowed to stand at room temp. for 1 hr, and then the upper phase of the mixture was washed 2 to 3 times with the PBS at 4°C. The leucocyte (neutrophil:lymphocyte:monocyte:basophil=82:10:4:3:0:8) suspension was obtained from the phase.

Release of SRS-A and PGs

a) SRS-A assay: The rat PEC (containing 2–5×10^5 mast cells) and human leucocyte suspension was incubated at 37°C for 5 min before the addition of 0.5 and μg/ml of ionophore A23187 (Calbiochem, San Diego, CA, U.S.A.), respectively, and then incubated for 10 min. The reaction mixture was cooled in ice and centrifuged at 180×g for 10 min to remove any sediment. An aliquot of the separated supernatant was applied to the isolated guinea-pig ileum in the presence of 10^{-7} g/ml of atropine and 10^{-6} g/ml of mepyramine. LTC4 release from rat PMC (containing 10^6 mast cells) by the addition of 300 μg/ml of DNP-Ascaris or 0.5 μg/ml of ionophore A23187 was measured using a Leukotriene C4-RIA Kit (New England Nuclear, Boston, MA, U.S.A.).

b) PGs assay: The suspensions of PMC and PEC (both containing 3×10^5 mast cells) were incubated at 37°C for 10 min before the addition of 300 μg/ml of DNP-Ascaris and 0.5 μg/ml of ionophore A23187, respectively, and then further incubated for 5 min. The reaction was stopped by 10^{-5} M of indomethacin and cooled in ice. PGE2 and 6-keto-PGE2 in the medium were extracted by the method of Inagawa et al. (13) and measured by radioimmunoassay (RIA).

c) Histamine assay: The suspension of rat PMC (2–5×10^5 mast cells) was incubated at 37°C for 5 min before adding 300 μg/ml of DNP-Ascaris and then incubated for 20 min. The reaction mixture was cooled in ice and centrifuged. The separated supernatant was mixed with 4% of HClO4 and boiled for 3 min. The mixture was then centrifuged and a clear supernatant was obtained. The histamine content was measured by the spectrofluorometric assay of Shore et al. (14).

Release of 3H-AA and its 3H-metabolites

Rat PMC (3×10^5) were incubated with 3H-AA (New England Nuclear, 10 μCi/10^7 cells) for 30 min at 37°C. The labeled cell suspension was centrifuged at 35×g for 10 min 4°C and washed 3 times with the medium. After the equilibration for 30 min at 37°C, again, the cell suspension was centrifuged and washed as described above. Labeled rat PMC was preincubated and added with 300 μg/ml of DNP-Ascaris. The reaction was stopped with 10% TCA. The supernatant from the reaction mixture was extracted with ethanol and chloroform as described by Rouzer et al. (15). The resulting organic solvent phase was dried by N2 gas, and the residue was developed by TLC using ethylacetate : iso-octane : acetic acid : methanol :
water (90:50:20:20:100). The radioactivities of $^3$H-AA, $^3$H-PGE$_2$, $^3$H-PGF$_{2\alpha}$ and $^3$H-PGD$_2$ on TLC were measured.

**Measurement of activities of 5-lipoxygenase, cyclooxygenase, PGI$_2$ synthetase and glutathione-S-transferase**

5-Lipoxygenase activities were measured using rat basophilic leukemia (RBL-1, Dai-nippon seiyaku Laboratory Products, Suita, Japan) and 1-$^{14}$C-AA as described by Jakschik and Lee (16). The radioactivities of $^{14}$C-AA and $^{14}$C-5-hydroxyeicosatetraenoic (5-HETE) were measured for the estimation of 5-lipoxygenase activity.

Cyclooxygenase activity was followed as described by Yoshimoto et al. (17). 1-$^{14}$C-AA (1 nmol, $5 \times 10^4$ cpm, New England Nuclear) and sheep vesicular gland microsomes (Ran Biochem., Tel Aviv, Israel) were incubated at 24°C for 1 min. The total radioactivities of $^{14}$C-PGE$_2$, $^{14}$C-PGF$_{2\alpha}$ and $^{14}$C-PGD$_2$ produced were measured as cyclooxygenase activity.

PGI$_2$ synthetase activity was measured using rabbit aorta microsomes and 1-$^{14}$C-PGHS which was produced from the reaction of 1-$^{14}$C-AA and sheep vesicular gland microsomes, as described by Watanabe et al. (18). The radioactivity of 6-keto-PGF$_{1\alpha}$ produced was measured as described by Yoshimoto et al. (17).

Glutathione-S-transferase activity was measured using human leucocytes which were prepared from the citrated blood and 4% dextran dissolved in 0.9% saline solution and LTA$_4$ (Funakoshi, Tokyo, Japan) in the presence of 1 mM of glutathione. The activity of LTC$_4$ produced was bioassayed with the isolated guinea-pig ileum.

**Test drugs**

Tranilast (Kissei, Matsumoto, Japan), disodium cromoglycate (DSCG, Fujisawa, Osaka, Japan), aspirin (Sanko, Tokyo, Japan) and mepacrine (Sigma, St. Louis, MO, U.S.A.). Tranilast was dissolved in 1% NaHCO$_3$, DSCG and mepacrine was dissolved in the buffered solution. Aspirin was dissolved in 99.5% ethanol. These drugs were diluted to the required concentrations by the buffered solution. Test drugs were applied to rat PMC, rat PEC and human leucocytes 1 min before the addition of antigen or ionophore A23187.

**Results**

**Effect of tranilast on the SRS-A release:** Figure 1 shows the inhibitory effect of tranilast and DSCG on the SRS-A release from rat PEC and human leucocytes by ionophore A23187. Tranilast at concentrations of $10^{-5}$ to $10^{-3}$ M suppressed the SRS-A release from both preparations in a dose-dependent manner. The IC$_{50}$ (the concentration which gives 50% inhibition) is approx. $10^{-4}$ M. However, DSCG hardly inhibited the SRS-A release from human leucocytes at concentrations of $10^{-6}$ to $10^{-3}$ M.

**Effect of tranilast and aspirin on the release of PGE$_2$ and 6-keto-PGF$_{1\alpha}$:** Figure 2 shows the inhibitory effect of tranilast on the release of PGE$_2$ from sensitized rat PMC by 300 μg/ml of DNP-Ascaris. When the antigen was added, the release of PGE$_2$ increased 2.7 times compared with the spontaneous release without the antigen. Tranilast at a concentration of $10^{-3}$ M markedly inhibited the release of PGE$_2$.

The release of PGE$_2$ and 6-keto-PGF$_{1\alpha}$ from rat PEC by 0.5 μg/ml of ionophore A23187.
A23187 increased to 5 times in the former and 2 times in the latter compared with the spontaneous release without the ionophore (Table 1). As shown in Table 1, tranilast at concentrations of $10^{-5}$ to $10^{-3}$ M inhibited the release of PGs in a dose-dependent manner, and $10^{-3}$ M of aspirin obviously suppressed it.

**Effect of tranilast on the release of $^3$H-AA and its $^3$H-metabolites:** As shown in Fig. 3, tranilast at concentrations of $10^{-5}$ to $10^{-3}$ M inhibited the release of $^3$H-AA, $^3$H-PGE$_2$, $^3$H-PGF$_{2\alpha}$ and $^3$H-PGD$_2$ from sensitized rat PMC by DNP-Ascariis markedly increased compared with the spontaneous release. Tranilast ($10^{-5}$-$10^{-3}$ M), DSCG ($10^{-6}$-$10^{-4}$ M) and mepracrine ($10^{-3}$ M) obviously inhibited the releases of $^3$H-AA and its $^3$H-metabolites.

**Effect of tranilast on the activities of 5-lipoxygenase, cyclooxygenase, PG1$_2$ synthetase and glutathione-S-transferase:** Figure 4 shows the effect of tranilast on the activities of 5-lipoxygenase, cyclooxygenase, PG1$_2$ synthetase and glutathione-S-transferase. Tranilast hardly affected the activities of these enzymes at concentrations of $10^{-5}$ to $10^{-3}$ M.

**The time course of release of histamine and LTC$_4$:** As shown in Fig. 5, the histamine release from rat PMC by ionophore A23187, initiated prior to LTC$_4$ release, reached approx. maximum level at 1 min after the addition of ionophore. On the other hand, that of LTC$_4$ reached maximum at 5 min. Similar results were obtained during antigen-antibody reactions using sensitized rat PMC (data not shown).

**Discussion**

SRS-A, oxidative metabolites of AA, is composed of LTC$_4$, LTD$_4$ and LTE$_4$, AA dissociated by the action of phospholipase A$_2$ from mast cell membrane phospholipid is metabolized by two major enzyme systems. The 5-lipoxygenase pathway eventually leads to the production of LTB$_4$, one of the most

---

**Table 1. Effects of tranilast and aspirin on the release of arachidonic acid metabolites (PGE$_2$ and 6-keto-PGF$_{1\alpha}$) from the rat peritoneal exudate cells**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Concentration (M)</th>
<th>Concentration in the medium (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PGE$_2$</td>
</tr>
<tr>
<td>Spontaneous</td>
<td></td>
<td>5.60$^a$</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>25.69±2.30$^b$</td>
</tr>
<tr>
<td>Tranilast</td>
<td>$10^{-5}$</td>
<td>24.35±1.14</td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>15.99±2.03$^*$</td>
</tr>
<tr>
<td></td>
<td>$10^{-3}$</td>
<td>14.19±3.62$^*$</td>
</tr>
<tr>
<td>Aspirin</td>
<td>$10^{-3}$</td>
<td>6.70±3.77$^{**}$</td>
</tr>
</tbody>
</table>

The rat peritoneal exudate cells were challenged with 0.5 μg/ml of ionophore A23187. Each value indicates the mean±standard errors of 3 to 5 experiments. $^a$: the mean of 2 experiments, $^b$: significant difference from the spontaneous release at $P<0.01$. $^*$ and $^{**}$: significant difference from the control at $P<0.05$ and $P<0.01$, respectively.
potent chemotactic molecules known, and SRS-A containing LTC₄, LTD₄ and LTE₄, while the cyclooxygenase pathway is responsible for the production of primary PGs, thromboxanes and prostacyclin (precursor of 6-keto-PGF₁α). Tranilast suppressed SRS-A release from rat PEC and human leucocytes by the addition of ionophore A23187 in a dose-dependent manner. In addition, prostaglandins (PG E₂ and/or 6-keto-PGF₁α), released by the addition of antigen and ionophore A23187, was markedly inhibited by tranilast in rat PEC and PMC. The release of labeled AA incorporated into mast cells was suppressed by tranilast and mepacrine (phospholipase A₂ inhibitor). Tranilast had no effect on activities of AA-converting enzymes such as 5-lipoxygenase, cyclooxygenase, glutathione-S-transferase and PG₁₂ synthetase. From these results, we speculate that tranilast may suppress the dissociation of AA from membrane phospholipids.

Fig. 3. Effects of tranilast, DSCG and mepacrine on the release of ³H-arachidonic acid (AA) and its ³H-metabolites from the sensitized purified rat mast cells challenged with 300 μg/ml of DNP-Ascaris. Each value indicates the mean of 3 experiments. Vertical bars show the standard errors of the mean. *, ** and ***: significant difference from the control at P<0.05, P<0.01 and P<0.001, respectively.

Fig. 4. Effect of tranilast on the activities of metabolizing enzymes in arachidonic acid cascades. Each value is expressed as a percentage of the control reaction and indicates the mean of 3 to 4 experiments. (○): cyclooxygenase, (■): PG₁₂ synthetase, (□): 5-lipoxygenase, (▲): glutathione-S-transferase.
phospholipase A₂ even at a concentration of $10^{-3}$ M. From these results, we suggest that the mechanism for the inhibitory action of tranilast on AA metabolism may be related to a process occurring before the dissociation of AA which is cleaved from membrane phospholipids.

Attention has been given to SRS-A and PGs as substances capable of modulating antigen-induced histamine release in a number of systems. The effect of AA and its metabolites on the histamine release from mast cells or basophils has been reported (19–25). PGD₂ preferentially released from mast cells by the addition of antigen and ionophore (26) does not have any influence on the histamine release (19). The PGE and PGF series do not have an inhibitory effect on the histamine release (20). In addition, indomethacin, a cyclooxygenase inhibitor, suppresses PGs production but has no influence on the histamine release from rat mast cells and basophil leukemia (RBL)-2H; conversely, it enhances the histamine release in human basophils (21, 22). On the other hand, eicosatetraynoic acid (ETYA) which blocks both lipoxygenase and cyclooxygenase inhibits the histamine release from rat mast cells and human leucocytes (23–25). The phospholipase A₂ inhibitors suppress production of AA and its metabolites and result in a decrease in histamine release (27, 28). The suppression of chemical mediator release by phospholipase A₂ inhibitors seems to be due to an indirect suppression of a lipoxygenase pathway through the inhibition of phospholipase A₂ activity. However, LTC₄ and LTD₄ have no influence on the histamine release (23, 25). In addition, neither the IgE-dependent release of SRS-A nor ionophore-induced SRS-A release initiate until the histamine release is almost complete (reference 1 and Fig. 5). Therefore, it is unlikely that metabolites catalyzed through the 5-lipoxygenase pathway as well as the cyclooxygenase pathway from AA are involved in the mechanisms of the histamine release from mast cells.

We demonstrated that tranilast at the same concentration inhibits the release of SRS-A and PGs not only in the antigen-antibody reaction but also in non-immunological stimulation. The concentration required to inhibit mediator release of tranilast in the present study coincides with that used in the previous studies in which the drug suppresses IgE-dependent histamine or SRS-A release from rat PEC and guinea-pig lung (5–11). Therefore, we assume that tranilast inhibits via a common process or through the
same process in the release of chemical mediators from mast cells.

It is noted that intracellular cyclic AMP and Ca ion mobilization play an important role in release of chemical mediators (1). Therefore, we think that it is necessary to investigate the effect of tranilast on cyclic AMP and Ca ion of mast cells in the future.

Acknowledgment: We thank Prof. A. Koda for pertinent advice and supervision of this manuscript.

References
8 Nakazawa, M., Yoshimura, T., Naito, J. and Azuma, H.: Pharmacological properties of N-(3',4'-dimethoxy-cinnamoyl) anthranilic acid (N-5'), a new anti-atopic agent. (5). Influence of N-5' on histamine release from peritoneal exudate cells. Folia PharmacoL Japon. 74, 483-490 (1978) (Abs. in English)
10 Komatsu, H., Ujiie, A. and Naito, J.: Effect of tranilast on the release of slow reacting substance of anaphylaxis (SRS-A) and on its contraction of smooth muscles. Folia Pharmacol. Japon. 82, 47-55 (1983) (Abs. in English)


