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# Tranilast inhibits contraction of rat aortic smooth muscle

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**Abstract**

Recently, the anti-allergic drug tranilast has been shown to reduce the rate of coronary restenosis after percutaneous transluminal coronary angioplasty. In this study, we investigated the effect of tranilast on contraction of and $Ca^{2+}$ movement in vascular smooth muscle. We measured the isometric force and fura-2-estimated intracellular $Ca^{2+}$ concentrations ($[Ca^{2+}]_i$) of rat aortic strips. Exposure of aortic strips to tranilast (0–500 μM) dose-dependently inhibited endothelin-1-induced increases in tension and $[Ca^{2+}]_i$ elevation of the strips. Similar inhibition by tranilast was observed in response to high $K^+$ stimulation. These results suggest that tranilast inhibits the contraction of vascular smooth muscle by inhibiting $Ca^{2+}$ mobilization, which might be related to its preventive effect on coronary restenosis after percutaneous transluminal coronary angioplasty. © 1997 Elsevier Science B.V.

*Keywords:* Tranilast; Muscle contraction; Restenosis; Angioplasty

## 1. Introduction

Percutaneous transluminal coronary angioplasty is being used with increasing frequency to treat obstructive narrowing of the coronary circulation. Despite a significant increase in primary success, restenosis occurs in 30–40% of patients and has complicated the long-term success of this treatment. The advanced occlusive atherosclerotic lesions observed in humans are characterized by a marked fibroproliferative response involving large numbers of intimal smooth muscle cells, macrophages, and T lymphocytes (Libby et al., 1992). The detailed mechanism of restenosis is unknown; however, the proliferation and migration as well as the contraction of vascular smooth muscle cells might contribute to the development of restenosis, and several neurohumoral factors and cytokines such as angiotensin II, platelet-derived growth factor (PDGF) and endothelin-1 have been implicated (Ross, 1993; Simons et al., 1994). Recently, it was reported that levels of endothelin-1 were elevated in the human coronary sinus after angioplasty (Tahara et al., 1992). Endothelin-1 induces vasoconstriction and the expression and release of several proto-oncogenes and growth factors (Komuro et al., 1988; Hirata et al., 1989; Bobik et al., 1990).

Tranilast, an anti-allergic drug, has been clinically used for the treatment of not only patients with bronchial asthma, allergic rhinitis, and atrophic dermatitis (Azuma et al., 1976), but also those with keloid and hypertrophic scars (Isagi et al., 1987) (Fig. 1). It inhibits the release of chemical mediators and cytokines from various cells (Koda et al., 1976; Komatsu et al., 1988), accumulation of collagen (Suzawa et al., 1992) and cell proliferation (Tanaka et al., 1994). Recently a double-blind large-scale multicenter trial demonstrated the potential efficacy of tranilast in preventing restenosis after percutaneous transluminal coronary angioplasty (The TREAT Study Investigators, 1994; Ueda et al., 1995) as well as after directional coronary atherectomy (Kosuge et al., 1995). Tranilast has also been shown to suppress intimal hyperplasia in the rabbit balloon injury model (Fukuyama et al., 1996). In this study, we investigated the effects of tranilast on contraction of and $Ca^{2+}$ movement in rat aortic arteries induced by endothelin-1 and high $K^+$.

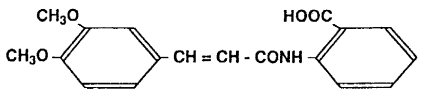


Fig. 1. Structure of tranilast.

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## 2. Materials and methods

### 2.1. Recording of mechanical responses

Male Sprague-Dawley rats (12–13 weeks old) were anesthetized by intraperitoneal administration of pentobarbital and killed by cervical dislocation. The thoracic aorta was excised and the surrounding tissue was mechanically removed. The aorta was opened helically and the endothelial cells were rubbed off with a cotton swab in oxygenated *N*-2-hydroxy-ethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) solution (in mM: NaCl 140; KCl 4.7; $CaCl_2$ 1.5; $NaH_2PO_4$ 1.0; $MgSO_4$ 1.0; HEPES 20; glucose 10, EDTA 0.03, ascorbic acid $0.5 \times 10^{-3}$). The preparations thus obtained were cut into spiral strips, 2–3 mm wide and 7–10 mm long. The strips were then mounted in 10 ml water-jacketed muscle chambers with sewing cotton thread, stretched to adjust the resting tension to 0.5 g and allowed to equilibrate for 60 min. Changes in isometric tension were measured with an isometric strain gauge transducer (TB611T, Nihon Kohden Kogyo, Tokyo, Japan) and displayed on a recorder with a built-in preamplifier (AP600G; Nihon Kohden Kogyo) as reported previously (Ohkawa et al., 1994).

### 2.2. Fura-2 loading and $[Ca^{2+}]_i$ measurement

The aortic strips were incubated in HEPES, 50 μM fura-2 pentaacetoxymethylester (fura-2/AM), 0.08% cremophore, and bubbled with a mixture of 95% $O_2$ and 5% $CO_2$ for 3 h at 15°C. The fura-2-loaded strips were washed with HEPES solution to remove the extracellular dye. The strips were then held horizontally on a silicon rubber sheet in a 4 ml organ bath maintained at 37°C. One end of the strips was pinned to the silicon rubber sheet and the other end was connected to an isometric force transducer (AP600G; Nihon Kohden Kogyo). Light emitted by fura-2 was detected with a CAF-100 $Ca^{2+}$ analyzer system (Nihon Bunko, Tokyo, Japan), and the analog output was displayed on a pen oscillograph (NEC San-ei, Tokyo, Japan) in nanoamperes. A part of the strip preparation was excited by light emitted from a xenon lamp (75 W) equipped with a rotating filter wheel (48 Hz) with 340 nm and 380 nm interference filters. The emitted light from the strips was collected into a photomultiplier through a 500 nm filter. The time constant of the optical channels was 1.0 s. The ratio of the fluorescence emitted from the aortic preparation which was excited at 340 nm and 380 nm was serially measured with the fluorimeter. The absolute values of intracellular $Ca^{2+}$ concentrations ($[Ca^{2+}]_i$) were calculated from the percent ratio ($R$), based on the following equation: $[Ca^{2+}]_i = K_d(R - R_{min})/(R_{max} - R)$ where $K_d$ is a dissociation constant and was assumed to be 224 nM (Grynkiewicz et al., 1985). $R_{max}$ was determined by addition of 25 μM ionomycin in physiological saline solution (PSS; 1.25 mM $Ca^{2+}$) and $R_{min}$ was determined in $Ca^{2+}$-free PSS (0 mM $Ca^{2+}$) containing 2 mM ethyleneglycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA). The isometric tension and $[Ca^{2+}]_i$ were recorded simultaneously.

### 2.3. Chemicals

Tranilast was kindly provided by Kissei Pharmaceutical (Matsumoto, Japan). Fura-2/AM was purchased from Dojindo (Kumamoto, Japan). Human endothelin-1 was purchased from Peptide Institute (Osaka, Japan). Other chemicals were of the highest grade commercially available.

### 2.4. Statistics

Results of experiments are expressed as means ± S.E.M. Comparisons were made by one-way analysis of variance (ANOVA) combined with Scheffé's test. $P$ values $< 0.05$ were regarded as significant.

## 3. Results

### 3.1. Effects of tranilast on contraction of rat aorta

Stimulation with endothelin-1 elicited a slowly developing, sustained contraction of the rat aorta, which generated a maximal force of 320 ± 32 mg at a concentration of $3 \times 10^{-8}$ M, followed by a sustained plateau of contraction. Pretreatment of rat aortic strips with tranilast (0–500 μM) for 30 min resulted in a dose-dependent reduction in the maximal force developed in response to endothelin-1 (Fig. 2). The contraction produced by endothelin-1 was decreased by 34% and 50% at doses of 100 and 500 μM tranilast, respectively.

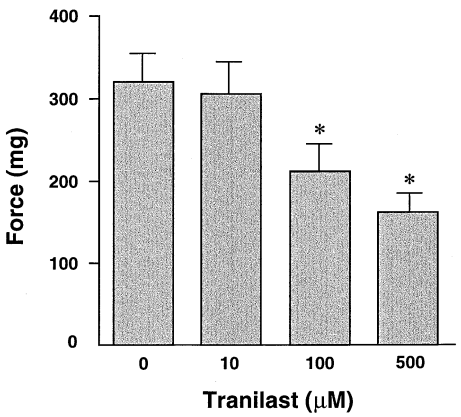


Fig. 2. Effects of tranilast on endothelin-1-induced contraction of rat aorta. Isometric forces in response to $3 \times 10^{-8}$ M endothelin-1 in the presence of various concentrations of tranilast (0–500 μM) were measured. Data are means ± S.E.M. of five experiments. * Significantly different from the control strips without tranilast.

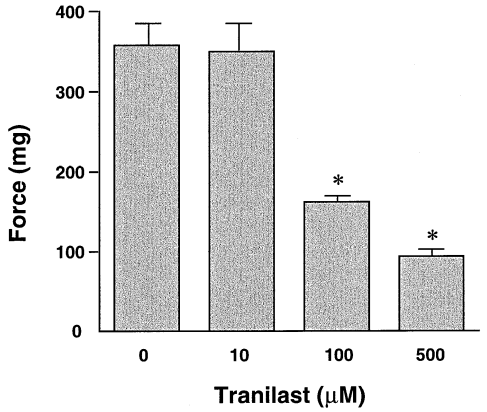


Fig. 3. Effects of tranilast on high $K^+$-induced contraction of rat aorta. Isometric forces in response to 50 mM KCl in the presence of various concentrations of tranilast (0–500 μM) were measured. Data are means ± S.E.M. of five experiments. * Significantly different from the control strips without tranilast.

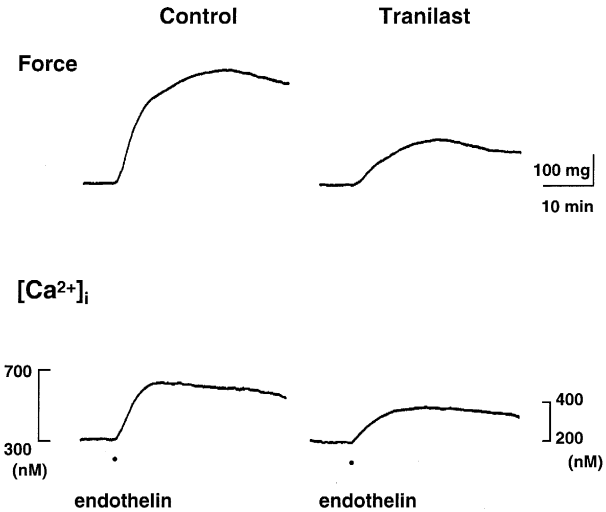


Fig. 4. Recordings showing the effects of tranilast on changes in $[Ca^{2+}]_i$ and tension induced by endothelin-1 in rat aortic strips. Tracings represent typical $[Ca^{2+}]_i$ (lower traces) and contractile (upper traces) responses to application of $3\times10^{-8}$ M endothelin-1 in the absence (left traces) or presence (right traces) of 100 μM tranilast.

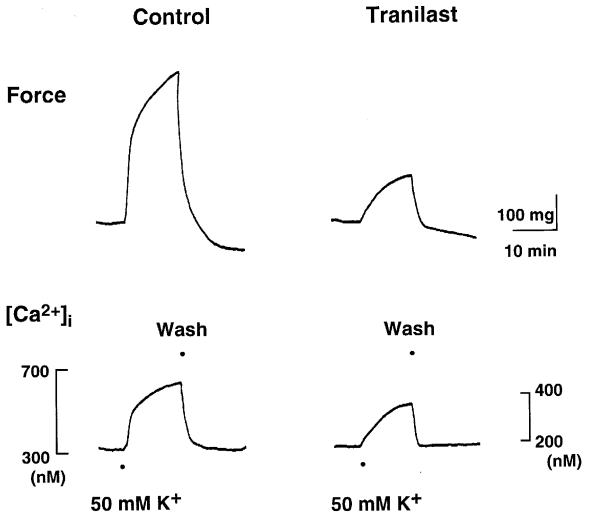


Fig. 5. Recordings showing the effects of tranilast on changes in $[Ca^{2+}]_i$ and tension induced by high $K^+$ in rat aortic strips. Tracings represent typical $[Ca^{2+}]_i$ (lower traces) and contractile (upper traces) responses to application of 50 mM KCl in the absence (left traces) or presence (right traces) of 100 μM tranilast.

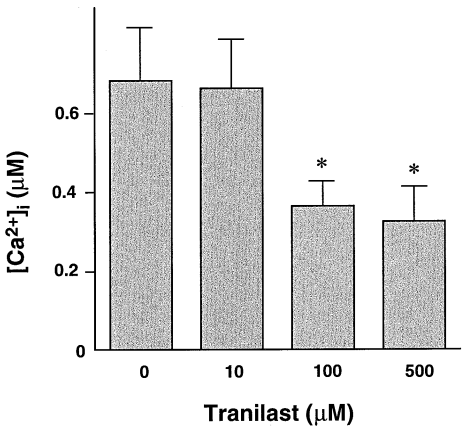


Fig. 6. Effects of tranilast on endothelin-1-induced $[Ca^{2+}]_i$ elevation. Peak $[Ca^{2+}]_i$ in response to $3\times10^{-8}$ M endothelin-1 in the presence of various concentrations of tranilast were measured. Data are means ± S.E.M. of four experiments. * Significantly different from the control strips without tranilast.

Table 1
Effects of tranilast on $[Ca^{2+}]_i$ and force of smooth muscle strips induced by endothelin-1 and high $K^+$

| | Endothelin-1 | | High $K^+$ | |
|---|---|---|---|---|
| | $[Ca^{2+}]_i$ (μM) | Force | $[Ca^{2+}]_i$ (μM) | Force |
| Basal | 0.31 ± 0.05 | 0 | 0.31 ± 0.06 | 0 |
| Peak | 0.71 ± 0.15 | 1.0 | 0.65 ± 0.14 | 1.0 |
| Tranilast | 0.20 ± 0.05 [a] | 0 | 0.21 ± 0.06 [a] | 0 |
| Tranilast + peak | 0.38 ± 0.08 [b] | 0.60 ± 0.09 [b] | 0.36 ± 0.07 [b] | 0.48 ± 0.10 [b] |

Values are means ± S.E.M. of six experiments. The aortic strips were treated (Tranilast) or not treated (Basal) with 100 μM tranilast for 30 min, and $3\times10^{-8}$ M endothelin-1 or 50 mM KCl was added. The maximum force induced by endothelin-1 or KCl in the absence of tranilast (Peak) was normalized as a relative force of 1.0 in each strip.

[a] Significantly different from Basal.

[b] Significantly different from Peak.

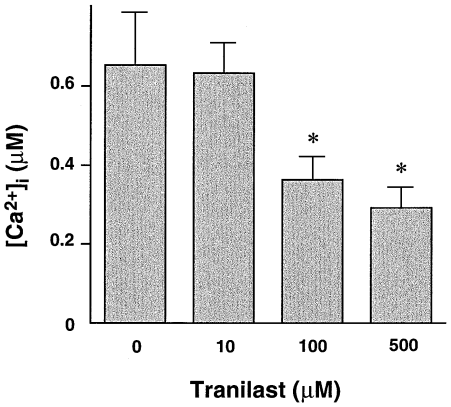


Fig. 7. Effects of tranilast on high $K^+$-induced $[Ca^{2+}]_i$ elevation. Peak $[Ca^{2+}]_i$ in response to 50 mM KCl in the presence of various concentrations of tranilast were measured. Data are means ± S.E.M. of four experiments. * Significantly different from the control strips without tranilast.

Exposure of the aortic strips to 50 mM KCl caused a rapid increase in the tension of the strips. The same dose of tranilast that depressed the rat aortic response to endothelin-1 also showed a significant suppressive effect on force development in response to depolarization with high $K^+$ (Fig. 3).

### 3.2. Effects of tranilast on changes in $[Ca^{2+}]_i$ and force

Fig. 4 and Table 1 show the effect of tranilast on changes in $[Ca^{2+}]_i$ and force induced by $3 \times 10^{-8}$ M endothelin-1 in fura-2-loaded smooth muscle strips. Stimulation with endothelin-1 showed a slow developing, sustained increase in $[Ca^{2+}]_i$. Pretreatment of rat aortic strips with tranilast (100 μM) significantly decreased the basal $[Ca^{2+}]_i$ and endothelin-1-induced $[Ca^{2+}]_i$ elevation.

On the other hand, stimulation with 50 mM KCl caused a rapid tonic increase in fura-2-estimated $[Ca^{2+}]_i$ (Fig. 5, Table 1). Tranilast also significantly decreased $[Ca^{2+}]_i$ elevation induced by high $K^+$.

Figs. 6 and 7 show the contraction-response relationship for the effects of tranilast on $[Ca^{2+}]_i$. Endothelin-1- and high $K^+$-induced $[Ca^{2+}]_i$ elevation was reduced by tranilast in a dose-dependent manner.

## 4. Discussion

Restenosis following technically successful angioplasty remains a challenging problem in clinical practice. The trauma experienced by the endothelium during angioplasty is speculated to be the stimulus for the process of restenosis which involves smooth muscle cell proliferation, migration of these cells from the media to the intima and synthesis of extracellular matrix. Recently, it was shown in a clinical study in Japan that oral administration of tranilast (600 mg/day) for 3 months reduced the rate of restenosis after percutaneous transluminal coronary angioplasty as compared with controls (12.7% vs. 38.0%) (The TREAT Study Investigators, 1994; Ueda et al., 1995). A standard clinical dose of tranilast (600 mg/day) produces an approximate plasma concentration of 100 μM. In the present study, tranilast at this concentration significantly inhibited contraction of the rat aortic arteries in response to the vasoactive agent endothelin-1 and direct depolarization of the smooth muscle by high $K^+$. The strips showed recovery of contraction in response to endothelin-1 or high $K^+$ after removal of tranilast (data not shown). Thus, the observed effects of tranilast are specific pharmacological effects, and not the result of nonspecific cytotoxicity.

Previously, Tanaka et al. (1994) reported that tranilast inhibited the proliferation and migration of, and collagen synthesis by rat vascular smooth muscle cells, and suggested that the inhibitory effect of tranilast was due to modification of the autocrine secretion of growth factors by vascular smooth muscle cells or to an influence on the activity of growth factors. Miyazawa et al. (1996b) reported antiproliferative effects of tranilast on human vascular smooth muscle cells in culture. Recently, Miyazawa et al. (1996a) also reported that tranilast specifically antagonized angiotensin II-induced contraction of rabbit aortic strips but showed no effect on the contraction induced by norepinephrine or endothelin-1. They concluded that the site of action of tranilast was the angiotensin II receptor or a closely related site. On the other hand, in the present study, tranilast inhibited the contraction and $[Ca^{2+}]_i$ elevation of rat aortic arteries induced by endothelin-1 or high $K^+$. We also observed that tranilast inhibited the contraction of porcine coronary arteries induced by endothelin-1 or angiotensin II (unpublished data). At present, we do not know the reason for the discrepancy in the results.

Endothelin is a potent vasoconstrictor peptide produced by the vascular endothelium. The local production of endothelin leads to a paracrine short-term (e.g., contraction) or long-term (e.g., growth-promoting effect) regulation of vascular function. It is more than likely that the level of endothelin in the walls of blood vessels at the site of angioplasty is significantly increased in response to shear stress on the vascular endothelium (Tahara et al., 1992). Endothelin also stimulates the production of growth factors such as PDGF which is a potent mitogen of smooth muscle and connective tissue cells (Remuzzi and Benigni, 1993). It is widely accepted that binding of endothelin to vasoconstriction receptors causes activation of phospholipase C by a G protein-mediated mechanism, and thus production of inositol trisphosphate ($IP_3$), which releases internally stored $Ca^{2+}$. Depletion of the internal $Ca^{2+}$ stores promotes $Ca^{2+}$ influx by an as yet unclear mechanism. In the present study, tranilast inhibited the increase in $[Ca^{2+}]_i$ of the rat aorta induced by endothelin-1. The reduced $[Ca^{2+}]_i$ response in aortic strips pretreated with tranilast may result from (i) decreased expression of func-

tional endothelin receptors, (ii) depressed phospholipase C activation and $IP_3$ production caused by altered coupling between endothelin receptor and phospholipase C, or (iii) reduction in the releasable internal $Ca^{2+}$ and/or altered functions of $Ca^{2+}$ influx pathways. Since not only endothelin-1 but also high $K^+$-induced $[Ca^{2+}]_i$ elevation was significantly inhibited by tranilast, the latter of the above three mechanisms appears to be the most likely. Recently, Nie et al. (1996) reported that tranilast inhibited PDGF-induced DNA synthesis in rat vascular smooth muscle cells by blocking PDGF-mediated $Ca^{2+}$ entry. However, we cannot rule out the possibility that tranilast also modulates $Ca^{2+}$ sensitivity of vascular smooth muscle.

Contractile and proliferative responses share common receptor-mediated signal-transduction mechanisms (Davies and Hagen, 1994). Agonist-receptor interaction modulates ion fluxes, $Ca^{2+}$ mobilization and phosphoinositide turnover, all of which are important in both contractile and proliferative responses of vascular smooth muscle cells. Functional responses of vascular smooth muscle cells to vasoactive agonists are representative of stimulus-response coupling in these cells, and define many of the common extracellular signals, surface receptor systems and intracellular regulatory mechanisms. So contractile responses may be indirectly representative of other functional responses such as proliferation, migration, metabolism, repair and rearrangement of structural appearance. Significantly, Ardissino et al. (1991) showed that the occurrence of abnormal coronary vasoconstriction influenced the risk of restenosis after successful dilation by angioplasty.

In conclusion, the present study showed that tranilast inhibits endothelin-1- and high $K^+$-induced contraction of rat aortic arteries by inhibiting $Ca^{2+}$ mobilization. This action of tranilast might be related to its preventive effect on development of restenosis after percutaneous transluminal coronary angioplasty.

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