British Journal of Pharmacology (1997) 122, 1061–1066

Tranilast inhibits the proliferation, chemotaxis and tube formation of human microvascular endothelial cells *in vitro* and angiogenesis *in vivo*

[1]Masayuki Isaji, Hiroshi Miyata, Yoshiyuki Ajisawa, *Yasuo Takehana & *Nagahisa Yoshimura

Discovery Research, R & D, Kissei Pharmaceutical Co., Ltd, 4365-1 Kashiwabara, Hotaka, Minamiazumi, Nagano-Pref. 399–83, and *Department of Ophthalmology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390, Japan

1 First developed as an antiallergic drug, tranilast inhibits chemical mediator release from mast cells. In the present study, we examine the effects of tranilast on angiogenesis *in vitro* and *in vivo* and discuss the application of tranilast for angiogenic diseases.

2 Tranilast inhibited significantly the proliferation (IC_{50}: 136 μM, 95% confidence limits: 134–137 μM) and vascular endothelium growth factor (VEGF)-induced chemotaxis (IC_{50}: 135 μM, 95% confidence limits: 124–147 μM) of human dermal microvascular endothelial cells (HDMECs) at concentrations greater than 25 μg ml^{-1}. No toxicity to HDMECs measuring by LDH release and no inhibitory effects on metalloproteinase (MMP)-2 and MMP-9 activity were observed even at 100 μg ml^{-1} (306 μM).

3 Tube formation of HDMECs cultured on the matrigel as an *in vitro* angiogenesis model was inhibited by tranilast in a concentration-dependent manner. The IC_{50} value and 95% confidence limits were 175 μM and 151–204 μM, respectively.

4 *In vivo* angiogenesis was induced in mice by the subcutaneous injection of matrigel containing 30 ng ml^{-1} VEGF and 64 μg ml^{-1} heparin. Tranilast was administered orally twice a day for 3 days. Tranilast dose-dependently suppressed angiogenesis in the matrigel and a significant change was observed at a dose of 300 mg kg^{-1}.

5 These results indicate that tranilast is an angiogenesis inhibitor which may be beneficial for the improvement of angiogenic diseases such as proliferative diabetic retinopathy, age-related macular degeneration, tumour invasion and rheumatoid arthritis.

Keywords: Tranilast; angiogenesis; microvascular endothelial cells; vascular endothelial growth factor; proliferation; chemotaxis; tube formation

Introduction

Tranilast has been used clinically as an antiallergic drug for the systemic and topical treatment of bronchial asthma, atopic dermatitis and allergic conjunctivitis. The clinical effectiveness of tranilast depends on inhibition of the release of chemical mediators from mast cells and of hypersensitivity reactions (Azuma *et al*., 1976; Koda *et al*., 1976). We have reported that in addition to effects on mast cells, tranilast inhibits the proliferation of fibroblasts *in vitro* and consequently suppresses proliferative inflammation *in vivo* (Isaji *et al*., 1987). Subsequently, it was found that tranilast inhibits the release of transforming growth factor (TGF)-β, interleukin (IL)-1β, prostaglandin (PG) E_2 and IL-2 from human monocytes and macrophages (Yanagi *et al*., 1987; Suzawa *et al*., 1992). In addition, we found that tranilast inhibits the transformation of fibroblasts to myofibroblasts and their contraction *in vitro* and suggested that tranilast may improve the fibroblast- or myofibroblast-induced contraction of fibrotic tissue (Isaji *et al*., 1994). Tanaka *et al*. (1994) found that tranilast inhibits the proliferation of smooth muscle cells. Tranilast also inhibited proliferative reactions of smooth muscle tissue in animals models for restenosis after percutaneous transluminal coronary angioplasty (PTCA) (Fukuyama *et al*., 1996) and has been used clinically for the treatment of restenosis after PTCA (Kato *et al*., 1996).

In the present study, we examined the effects of tranilast on angiogenesis *in vitro* and *in vivo* and consider its use in treating other diseases concomitant with excess angiogenesis.

[1] Author for correspondence.

Methods

Cell culture

Human dermal microvascular endothelial cells (HDMECs, Cell System Corporation, WA, U.S.A.) were purchased from Dainippon Seiyaku Co., Ltd, Japan. The cells in microvascular endothelial cell (MvE) medium (Cell System Corporation) were plated on collagen-coated culture plates (Toyobo Engineering Co., Ltd, Japan) and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells from passage 3 to 5 were used.

MMP-2 and MMP-9 assay

Human matrix metalloproteinase (MMP)-2 and MMP-9 (biotechnological grade) were purchased from Yagai Research Center, Japan. MMP activity was determined by the degradation of fluorescein-labelled type IV collagen using a type IV collagenase assay kit (Yagai Research Centre, Japan). Tranilast (MW: 327.24, Kissei Pharmaceutical Co, Ltd, Japan) was solubilized with 1% $NaHCO_3$ solution at 70°C and diluted with assay buffer at a final concentration of 0, 12.5, 25, 50 and 100 μg ml^{-1}.

HDMEC proliferation

HDMECs in MvE medium were seeded on collagen-coated 6-well plates at 2×10^4 cells/plate. After 1 day, the medium was aspirated and replaced with the fresh medium containing various concentrations of tranilast (final concentrations: 0, 12.5, 25, 50 and 100 μg ml^{-1}). On day 2, the medium was aspirated and 0.25% trypsin containing 0.02% EDTA added to remove the cells from the plate. The number of cells was determined by a haemocytometer in five cultures. Viability of cells was assessed by measuring lactate dehydrogenase (LDH)

release as a marker of cell injury from HDMEC treated with tranilast for 2 days. LDH activity in medium and cells (lysed by adding 0.2% Tween 20) was determined using LDH CII Test Wako (Wako Pure Chemical Industries, Ltd, Japan). Released LDH was defined as the percentage of extracellular compared to total LDH activity.

HDMEC chemotaxis

Vascular endothelial growth factor (VEGF, Genzyme Corporation, U.K.)-induced chemotaxis of HDMEC (Ferrara & Davis-Smyth, 1997) was determined in a 96-well microchemotaxis chamber (Neuro Probe Inc., U.S.A.). A polycarbonate filter of 10 μm thickness with 8 μm pore size was coated with 50 μg ml^{-1} type 1 collagen (Cellmatrix Type I-P, Nitta Gelatin, Japan). HDMECs (50 μl of 2×10^4 cells ml^{-1}) in Dulbecco's modified Eagle medium (DMEM) and Ham's F12 (1:1) containing 0.1% bovine serum albumin (BSA) and various concentrations of tranilast were applied in the upper compartment of the chamber. The medium (32 μl) containing 100 ng ml^{-1} VEGF, 0.1% BSA, and tranilast was added to the lower compartment of the chamber. The chamber was incubated at 37°C for 5 h in 5% CO_2 and 95% atmosphere. After incubation, the filter was removed, and the migrated cells were fixed with 90% ethanol and stained with Diff-Quick (Baxter Diagnostics Inc.). The number of migrated cells was counted in five random fields at $\times400$ magnification under phase-contrast microscopy.

In vitro *tube formation*

According to the method described by Haralabopoulos *et al.* (1994), 250 μl matrigel (10 mg protein ml^{-1}, Becton Dickinson Labware, MA, U.S.A.) was added to a 24-well plate (Corning, U.S.A.) and was then allowed to solidify at 37°C for 1 h. HDMECs (1×10^4 cells) were seeded on the matrigel and cultured in the MvE medium containing various concentrations of tranilast at 37°C for 18 h in a humidified atmosphere of 5% CO_2 in air. After incubation, five different fields in six cultures were randomly observed with a phase-contrast microscope and photographed at $\times40$ magnification. The lengths of the tube structures were measured.

In vivo *angiogenesis*

Nine-week-old male C57BL/6 mice were purchased from SLC, Japan. According to the method described by Passaniti *et al.* (1992), 0.5 ml matrigel (10 mg protein ml^{-1}) containing VEGF (30 ng ml^{-1}) and heparin (64 units ml^{-1}, Novo Nordisk) was injected s.c. on the dorsal surface. Tranilast was dissolved in 0.5% carboxymethyl cellulose-Na (CMC-Na, Nacalai Tesque, Japan) and administered orally twice a day for 3 days. Three days later, the mice were sacrificed under ether anaesthesia, and matrigel was harvested from surrounding connective tissue. After digestion by 0.02% collagenase, the haemoglobin content of the matrigel was determined by Hemoglobin Test Wako (Wako Pure Chemical Industries Ltd, Japan).

Table 1 Effects of tranilast on MMP-2 activity and MMP-9 activity

Tranilast (μg ml^{-1})	*MMP-2 activity* (unit ml^{-1})	*MMP-9 activity* (unit ml^{-1})
0	1.09±0.07 (100)	2.49±0.16 (100)
12.5	1.07±0.07 (98)	2.51±0.06 (101)
25.0	1.05±0.07 (96)	2.66±0.05 (107)
50.0	1.03±0.07 (95)	2.56±0.07 (103)
100.0	1.07±0.06 (98)	2.54±0.09 (102)

MMP activity was determined by measuring the fluorescent intensity released from fluorescein-labelled type IV collagen substrate by human MMP-2 or MMP-9. Units of MMP activity are defined as μg of substrate degraded per min. Numbers in parentheses represent mean percentage of the control. Data are shown as means±s.e.mean of five samples.

Statistical analysis

Data are expressed as means±s.e.mean. Analysis of the regression line test was used to calculate IC_{50} or ED_{50} and their 95% confidence limits. Statistical significance between groups was performed by one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered statistically significant at $P<0.05$.

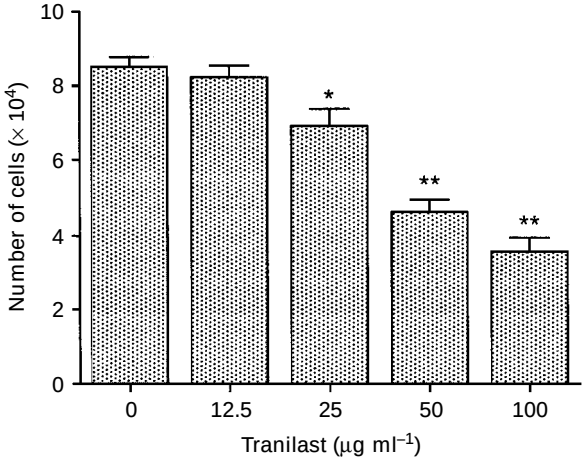

Figure 1 Effects of tranilast on proliferation of HDMECs. HDMECs (2×10^4) were incubated at 37°C in MvE growth medium. After incubation for 1 day, medium was aspirated and the fresh medium containing various concentrations of tranilast was added to the cells and incubated for a further 2 days. The number of cells was determined in five cultures by a haemocytometer. Data are the means±s.e.mean. Statistically significant difference compared with responses in the absence of tranilast, $*P<0.05$, $**P<0.01$.

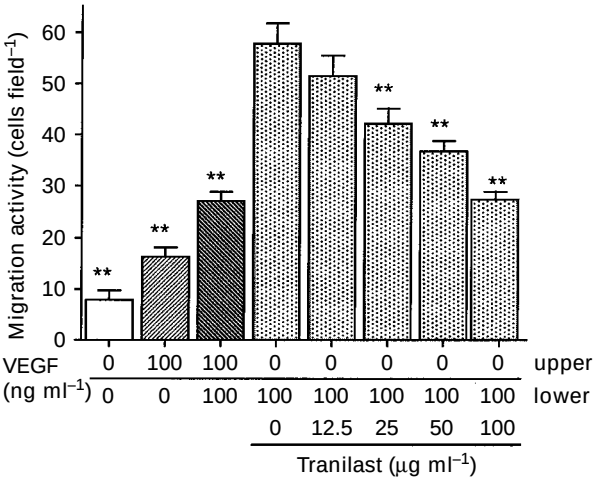

Figure 2 Effects of tranilast on VEGF-induced chemotaxis of HDMECs. VEGF-induced chemotaxis of HDMECs was determined by the Boyden chamber method using collagen-coated membrane (8 μm pore size). HDMECs (2×10^4) in DMEM and Ham's F12 (1:1) containing 0.1% BSA were placed in the upper chamber. VEGF was included in upper and lower chambers. The chamber was incubated at 37°C for 5 h. The number of cells migrating to the lower side of the membrane was counted in five random fields at $\times400$ magnification under phase-contrast microscopy. Data are the means ±s.e.mean. Statistically significant difference compared to responses in the absence of tranilast, $**P<0.01$.

Results

MMP-2 and MMP-9 activity

The results on MMP-2 and MMP-9, which are related to the degradation of basement membrane in the process of angiogenesis, are shown in Table 1. MMP-2 activity and MMP-9 activity were slightly decreased and increased by the treatment of tranilast compared to controls, respectively. However, those changes were less than 10% of control values and no significant changes were observed. Tranilast did not affect MMP-2 and MMP-9 activity even at a concentration of 100 μg ml^{-1} (306 μM).

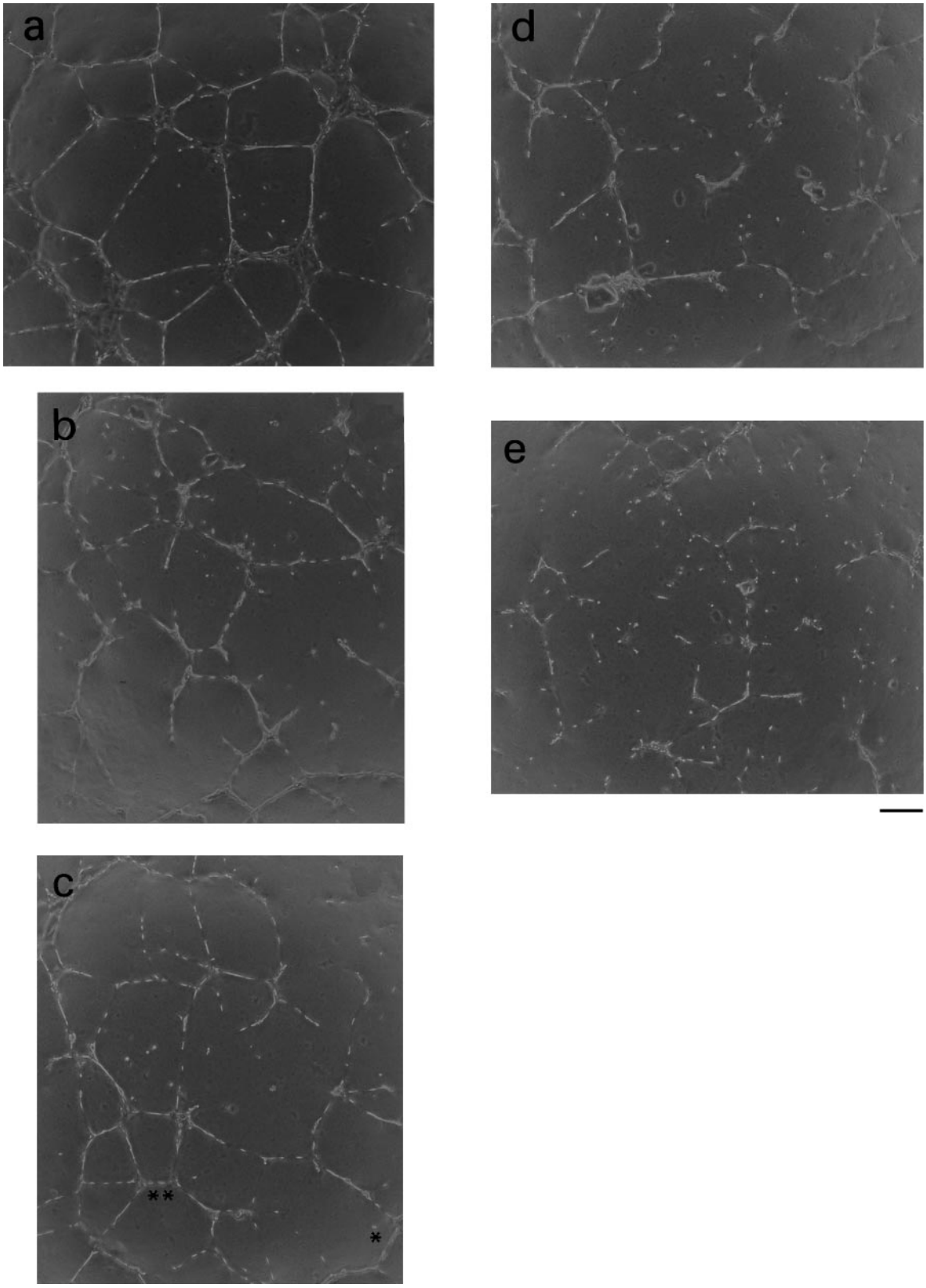

Figure 3 Phase-contrast micrographs of tube formation of HDMECs on matrigel. HDMECs were seeded on matrigel and incubated at 37°C for 18 h in medium containing various concentrations of tranilast. Morphological features of HDMECs treated with medium alone (a), 12.5 μg ml^{-1} (b), 25 μg ml^{-1} (c), 50 μg ml^{-1} (d) or 100 μg ml^{-1} tranilast (e) were photographed at $\times 40$ magnification with phase-contrast microscopy. Bar = 100 μm.

HDMEC proliferation

As shown in Figure 1, proliferation of HDMECs was inhibited by tranilast in a concentration-dependent manner. Significant differences were observed at a tranilast concentration of 25 μg ml^{-1}. IC_{50} value and 95% confidence limits were 44.3 μg ml^{-1} (136 μM) and 43.8–44.9 μg ml^{-1} (134–137 μM), respectively. The effect of tranilast on LDH release, which is a marker of cell injury, from HDMECs was assessed. Tranilast exerted no significant effects on the viability of cells even at 306 μM (LDH release: 17.8$\pm$3.1%) compared to the control (LDH release: 16.6$\pm$3.1%). Thus, selective effects of tranilast on inhibition of HDMECs proliferation without affecting cell viability were observed.

HDMEC chemotaxis

Addition of VEGF (100 ng ml^{-1}), which has an important role for angiogenesis (Ferrara & Davis-Smyth, 1997), in only the lower chamber markedly increased HDMEC migration by approximately 3.4 and 2.1 fold compared to the addition of VEGF in only the upper chamber and in both upper and lower chambers, respectively (Figure 2). Thus, chemotaxis of HDMECs depended on the gradient of VEGF concentration. The VEGF-induced chemotaxis (addition of VEGF in the only lower chamber) was significantly inhibited by tranilast at the same concentrations (>25 μg ml^{-1}) that inhibited the proliferation of HDMECs (Figure 2). The IC_{50} value and 95% confidence limits were 44.1 μg ml^{-1} (135 μM) and 40.5–48.1 μg ml^{-1} (124–147 μM), respectively.

In vitro *tube formation*

As an *in vitro* angiogenesis model, we used the tube formation of HDMECs on matrigel and examined the effects of tranilast on *in vitro* angiogenesis. As shown in Figure 3a, HDMECs were elongated and appeared as a network formation on the matrigel. As the concentration of tranilast was increased, the tube formation was visibly reduced (Figure 3b–e). The result of quantitative measurement of the tube length is shown in Figure 4. Treatment with tranilast reduced the tube formation of HDMECs in a concentration-dependent manner. Significant inhibition was observed at concentrations greater than 25 μg ml^{-1}, and the IC_{50} value and 95% confidence limits were 57 μg ml^{-1} (175 μM) and 49.2–66.5 μg ml^{-1} (151–204 μM), respectively.

In vivo *VEGF-induced angiogenesis*

As VEGF has been recognized as an important angiogenic factor, *in vivo* angiogenic reactions were induced by the s.c. injection of matrigel containing heparin and VEGF. Effects of tranilast on angiogenesis were examined by measuring the haemoglobin content in the matrigel. In a dose-dependent manner, tranilast suppressed the VEGF-induced angiogenesis in matrigel; 58% of significant suppression was observed at a dose of 300 mg kg^{-1} (Figure 5). The ED_{50} value and 95% confidence limits were 165 mg kg^{-1} and 162–169 mg kg^{-1}, respectively.

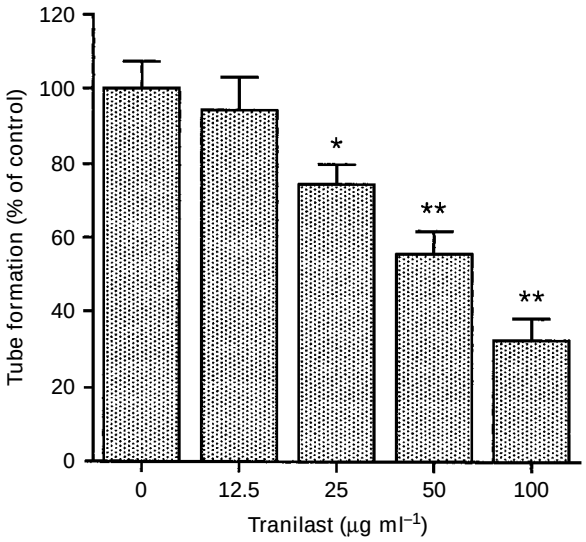

Figure 4 Effects of tranilast on tube formation of HDMECs on matrigel. HDMECs (1×10^4) were seeded onto the matrigel and incubated at 37°C in MvE growth medium containing various concentrations of tranilast. After 18 h, tube formation on the matrigel was photographed in five random fields at $\times$40 magnification under phase-contrast microscopy. The length of tube structures was measured and expressed as percent of control (0 μg ml^{-1} tranilast). Data are the means$\pm$s.e.mean of six cultures. Statistically significant difference compared with responses in the absence of tranilast, $^*P<0.05$, $^{**}P<0.01$.

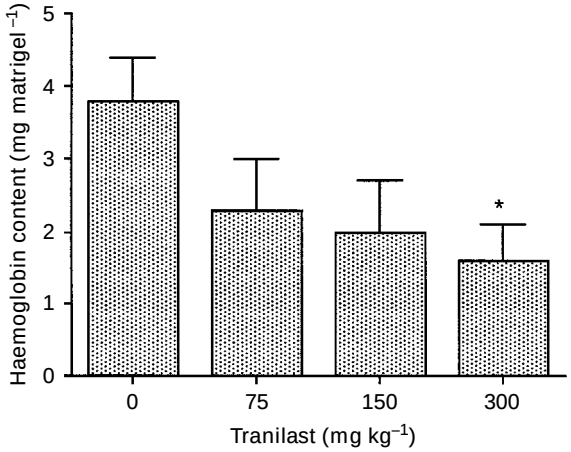

Figure 5 Effects of tranilast on VEGF-induced angiogenesis *in vivo*. Matrigel (10 mg protein ml^{-1}) containing 30 ng ml^{-1} VEGF and 64 units ml^{-1} heparin was injected s.c. in mice. Tranilast was dissolved in 0.5% carboxymethylcellulose solution and administered orally twice a day for 3 days. The matrigel was harvested, and the haemoglobin content of the matrigel was determined as an index of angiogenesis. Data are shown as means$\pm$s.e.mean from eight mice. Statistically significant difference, compared to responses in the absence of tranilast, $^*P<0.05$.

Discussion

Angiogenesis involves destruction of the basement membrane by proteases, migration and proliferation of endothelial cells, and transformation of endothelial cells to form tubes (Battegay, 1995). To suppress excess angiogenesis, collagenase inhibitors (Ray & Stetler-Stevenson, 1996) and inhibitors of migration, proliferation and transformation of endothelial cells have been developed (Ingber *et al*., 1990; Vukanovic *et al*., 1993; Hu & Fan, 1995; Seed, 1996). We found that tranilast inhibits important steps of angiogenesis including chemotaxis, proliferation and tube formation of microvascular endothelial cells *in vitro*, and exerts an inhibitory effect on angiogenesis *in vivo*. At effective concentrations and doses, tranilast did not affect cell viability as determined by measuring LDH release from HDMECs, and mouse growth, so its effects on angiogenesis are considered to be selective.

Among the angiogenic factors examined, VEGF has been shown to play a key role in angiogenesis (Ferrara & Davis-Smyth, 1997) and it is therefore important to assess the responses of endothelial cells to VEGF. Tranilast inhibited VEGF-induced chemotaxis and subsequent proliferation of endothelial cells. Although the precise inhibitory mechanisms of tranilast in these reactions have not been elucidated, this

compound inhibits energy consumption and/or Ca^{2+} influx at the time of degranulation from mast cells (Koda *et al*., 1985). Energy consumption and Ca^{2+} also play important roles in the chemotaxis and proliferation of various cells (Lane & Lamkin, 1984; Henry, 1990), and so it is possible that tranilast acts to inhibit these processes in endothelial cells. Tranilast was reported to arrest the cell cycle at the G_0/G_1 phase (Isaji *et al*., 1994), and this may participate in the selective inhibition of cell proliferation without toxic effects. Antiproliferative substances affecting the S phase and/or M phase of the cell cycle cause cell death concomitantly with inhibition of proliferation and could, therefore, not have selective effects on proliferation. Tranilast thus appears to be an angiogenesis inhibitor affecting the G_0/G_1 phase of the cell cycle.

Degradation of extracellular matrix by MMPs, chemotaxis, proliferation and morphological differentiation of endothelial cells are involved in tube formation of endothelial cells on matrigel (Kubota *et al*., 1988). The effect of tranilast on tube formation may be due to effects on all these processes. However, tranilast did not affect MMP-2 or MMP-9 activity, which has important roles in degradation of the basement membrane (Fabunmi *et al*., 1996). It is known that tranilast inhibits the transformation of fibroblasts to myofibroblasts in culture (Isaji *et al*., 1994). Although it is difficult to evaluate, the effect of tranilast on the transformation of endothelial cells is an interesting subject for future research. Tranilast significantly inhibited VEGF-induced angiogenesis at 300 mg kg^{-1} *in vivo*. When 300 mg kg^{-1} of tranilast was administered p.o. in mice, the plasma concentration sufficient to reveal its *in vitro* effects was maintained for about 8 h (data not shown).

Tranilast has an inhibitory effect on mast cell degranulation (Azuma *et al*., 1976) and it has been reported that mast cells participate in angiogenesis (Rizzo & DeFouw, 1996). Mast cells produce angiogenic cytokines and secrete granules containing inflammatory mediators and heparin in response to inflammatory and allergic stimuli (Qu *et al*., 1995). Heparin binds to various angiogenic cytokines and enhances their angiogenic activities *in vitro* (Castellot *et al*., 1986) and *in vivo* (Norrby & Sorbo, 1992; Passaniti *et al*., 1992). Although the participation of mast cells in angiogenesis has not been clarified in detail, it is possible that tranilast inhibited a part of the *in vivo* angiogenesis enhanced by heparin released from mast cells. A dose of 300 mg kg^{-1} was sufficient to inhibit a hypersensitivity reaction dependent on mast cell degranulation (Azuma *et al*., 1976). Further studies are needed to clarify whether tranilast affects angiogenic cytokine release from mast cells and to what degree mast cells participate in angiogenesis *in vivo*.

Angiogenesis is involved in the pathogenesis of tumour invasion and rheumatoid arthritis (Folkman, 1995). In tumor metaplasia, degradation of basement membrane by MMPs is a key factor as well as angiogenesis and MMP inhibitors could suppress metastasis and tumour progression (Stetler-Stevenson *et al*., 1996). Many factors are related to the pathogenesis of rheumatoid arthritis, although these include proliferative inflammatory reactions concomitant with angiogenesis (Poole, 1993; Folkman, 1995). Further studies are needed to investigate the effects of tranilast in these clinical conditions. Angiogenesis is also a predominant feature of the pathogenesis in diabetic retinopathy and age-related macular degeneration. These diseases are characterised by angiogenesis, proliferation of fibrotic tissue and contraction of the fibrotic tissue, which cause clinical symptoms such as bleeding and destruction of the retina (Garner, 1993; Killingsworth, 1995). Tranilast has various pharmacological effects other than inhibition of angiogenesis including inhibition of fibroblast proliferation (Isaji *et al*., 1987), cytokine release from monocytes and macrophages (Yanagi *et al*., 1987; Suzawa *et al*., 1992), transformation of fibroblasts to myofibroblasts and their contraction (Isaji *et al*., 1994) and collagen deposition *in vivo* (Isaji *et al*., 1987, 1994). Thus, tranilast may exert its beneficial effects for the treatment of these two diseases by inhibiting angiogenesis and the development of fibrotic tissue around the microvascular vessels and their contraction.

In conclusion, tranilast is a novel angiogenesis inhibitor *in vitro* and *in vivo* concomitant with inhibitory effects on mast cell degranulation and proliferative inflammation. Depending on its pharmacological effects and clinical safety, tranilast may be beneficial for the treatment of angiogenic diseases, especially diabetic retinopathy and age-related macular degeneration.

We thank Ms Shiho Tanaka for assistance with analysis of tube formation.

References

AZUMA, H., BANNO, K. & YOSHIMURA, T. (1976). Pharmacological properties of *N*-(3′,4′-dimethoxycinnamoyl)anthranilic acid (*N*-5′), a new anti-atopic agent. *Br. J. Pharmacol*., **58,** 483–488.

BATTEGAY, E.J. (1995). Angiogenesis mechanistic insights, neovascular diseases, and therapeutic prospects. *J. Mol. Med*., **73,** 333–346.

CASTELLOT, J.J., KAMBE, A.M., DOBSON, D.E. & SPIEGELMAN, B.M. (1986). Heparin potentiation of 3T3-adipocyte stimulated angiogenesis: mechanism of action on endothelial cells. *J. Cell. Physiol*., **127,** 323–329.

FABUNMI, R.P., BAKER, A.H., MURRAY, E.J., BOOTH, R.F. & NEWBY, A.C. (1996). Divergent regulation by growth factors and cytokines of 95 kDa and 72 kDa gelatinases and tissue inhibitors or metalloproteinase-1, -2 and -3 in rabbit aortic smooth muscle cells. *Biochem. J*., **315,** 335–342.

FERRARA, N. & DAVIS-SMYTH, T. (1997). The biology of vascular endothelial growth factor. *Endocrinol. Rev*., **18,** 4–25.

FOLKMAN, J. (1995). Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Med*., **1,** 27–31.

FUKUYAMA, J., ICHIKAWA, K., MIYAZAWA, K., HAMANO, S., SHIBATA, N. & UJIIE, A. (1996). Tranilast suppresses intimal hyperplasia in the balloon injury model and cuff treatment model in rabbits. *Japn. J. Pharmacol*., **70,** 321–327.

GARNER, A. (1993). Histopathology of diabetic retinopathy in man. *Eye*, **7,** 250–253.

HARALABOPOULOS, G.C., GRANT, D.S., KLEINMAN, H.K., LELKES, P.I., PAPAIOANNOU, S.P. & MARAGOUDAKIS, M.E. (1994). Inhibitors of basement membrane collagen synthesis prevent endothelial cell alignment in matrigel *in vitro* and angiogenesis *in vivo*. *Lab. Invest*., **71,** 575–582.

HENRY, P.D. (1990). Antiatherogenic effects of calcium-channel blockers: possible mechanisms of action. *Cardiovas. Drugs Ther*., **4,** 1015–1020.

HU, D.E. & FAN, T.-P.D. (1995). Suppression of VEGF-induced angiogenesis by the protein tyrosine kinase inhibitor, lavendustin A. *Br. J. Pharmacol*., **114,** 262–268.

INGBER, D., FUJITA, T., KISHIMOTO, S., SUDO, K., KANAMARU, T., BREM, H. & FOLKMAN, J. (1990). Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumor growth. *Nature*, **348,** 555–557.

ISAJI, M., ARUGA, N., NAITO, J. & MIYATA, H. (1994). Inhibition by tranilast of collagen accumulation in hypersensitive granulomatous inflammation *in vivo* and of morphological changes and functions of fibroblasts *in vitro*. *Life Sci*., **55,** PL287–P292.

ISAJI, M., NAKAJOH, M. & NAITO, J. (1987). Selective inhibition of collagen accumulation by *N*-(3,4-dimethoxycinnamoyl)anthranilic acid (*N*-5′) in granulation tissue. *Biochem. Pharmacol*., **36,** 469–474.

KATO, K., TAMAI, H., HAYAKAWA, H., YAMAGUCHI, T., KANMATSUSE, K., HAZE, K., AIZAWA, T., SUZUKI, S., TAKASE, S., SUZUKI, T., NISHIKAWA, H., NAKANISHI, S., KATO, O. & NAKASHIMA, M. (1996). Clinical evaluation of tranilast on restenosis after percutaneous transluminal coronary angioplasty (PTCA). A double-blind placebo-controlled comparative study. *J. Clin. Ther. Med.*, **12,** 65–85.

KILLINGSWORTH, M.C. (1995). Angiogenesis in early choroidal neovascularization secondary to age-related macular degeneration. *Graefes Arch. Clin. Exp. Ophthalmol.*, **233,** 313–323.

KODA, A., NAGAI, H., WATANABE, S., YANAGIHARA, Y. & SAKAMOTO, K. (1976). Inhibition of hypersensitivity reactions by a new drug, *N*-(3′,4′-dimethoxycinnamoyl)anthranilic acid (*N*-5′). *J. Allergy Clin. Immunol.*, **57,** 396–407.

KODA, A., KURASHINA, Y. & NAKAZAWA, M. (1985). The inhibition mechanism of histamine release by *N*-(3,4-dimethoxycinnamoyl) anthranilic acid. *Int. Archs. Allergy Appl. Immun.*, **77,** 244–245.

KUBOTA, Y., KLEINMAN, H.K., MARTIN, G.R. & LAWLEY, T.J. (1988). Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. *J. Cell Biol.*, **107,** 1589–1598.

LANE, T.A. & LAMKIN, G.E. (1984). A reassessment of the energy requirements for neutrophil migration: adenosine triphosphate depletion enhances chemotaxis. *Blood*, **64,** 986–993.

NORRBY, K. & SORBO, J. (1992). Heparin enhances angiogenesis by a systemic mode of action. *Int. J. Exp. Pathol.*, **73,** 147–155.

PASSANITI, A., TAYLOR, R.M., PILI, R., GUO, Y., LONG, P.V., HANEY, J.A., PAULY, R.R., GRANT, D.S. & MARTIN, G.R. (1992). A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. *Lab. Invest.*, **67,** 519–528.

POOLE, A.R. (1993). Cartilage in health and disease. In *Arthritis and Applied Conditions*, ed. McCarty, D.J. & Koopman, W.J., Vol. 1, pp. 279–333. London: Lea & Febiger.

QU, Z., LIEBLER, J.M., POWERS, M.R., GALEY, T., AHMADI, P., HUANG, X.N., ANSEL, J.C., BUTTERFIELD, J.H., PLANCK, S.R. & ROSENBAUM, J.Y. (1995). Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *Am. J. Pathol.*, **147,** 564–573.

RAY, J.M. & STETLER-STEVENSON, W.G. (1996). Matrix metalloproteinases and malignant disease: recent developments. *Exp. Opin. Invest. Drugs*, **5,** 323–335.

RIZZO, V. & DEFOUW, D.O. (1996). Mast cell activation accelerates the normal rate of angiogenesis in the chick chorioallantoic membrane. *Microvasc. Res.*, **52,** 245–257.

SEED, M.P. (1996). Angiogenesis inhibition as a drug target for disease: an update. *Exp. Opin. Invest. Drugs*, **5,** 1617–1637.

STETLER-STEVENSON, W.G., HEWITT, R. & CORCORAN, M. (1996). Matrix metalloproteinases and tumor invasion: from correlation and causality to the clinic. *Semin. Cancer Biol.*, **7,** 147–154.

SUZAWA, H., KIKUCHI, S., ARAI, N. & KODA, A. (1992). The mechanism involved in the inhibitory action of tranilast on collagen biosynthesis of keloid fibroblasts. *Jpn J. Pharmacol.*, **60,** 91–96.

TANAKA, K., HONDA, M., KURAMOCHI, T. & MORIOKA, S. (1994). Prominent inhibitory effects of tranilast on migration and proliferation of and collagen synthesis by vascular smooth muscle cells. *Atherosclerosis*, **107,** 179–185.

VUKANOVIC, J., PASSANITI, A., HIRATA, T., TRAYSTMAN, R.J., HARTLEY-ASP, B. & ISAACS, J.T. (1993). Antiangiogenic effects of the quinoline-3-carboxamide linomide. *Cancer Res.*, **53,** 1833–1837.

YANAGI, T., WATANABE, M., FUKUDA, S. & TSUJI, Y. (1987). Suppressive effects of tranilast (TN) on human mononuclear cells. *Jpn J. Inflam.*, **7,** 169–173.

(Received February 18, 1997
Revised July 16, 1997
Accepted August 19, 1997)