Inhibition of Neointima Formation by Tranilast in Pig Coronary Arteries After Balloon Angioplasty and Stent Implantation

Sugao Ishiwata, MD, FACC,* Stefan Verheye, MD,* Keith A. Robinson, PhD, FACC,† Mahomed Y. Salame, MRCP,* Hector de Leon, MD, PhD,* Spencer B. King III, MD, FACC,* Nicolas A. F. Chronos, MD, FACC†

Atlanta, Georgia

OBJECTIVES
We evaluated the effect of orally administered tranilast, N-(3,4-dimethoxycinnamoyl)anthranilic acid, on histologic and histomorphometric changes after angioplasty or stent implantation in pig coronary arteries.

BACKGROUND
Tranilast, which has antikeloid and antiallergic properties and therefore may modulate the fibrotic and inflammatory tissue responses to angioplasty and stenting, has been shown to inhibit angiographic restenosis in small clinical trials. However, its effect on histomorphometric changes in coronary arteries after angioplasty and stenting is unknown.

METHODS
Following initial pharmacokinetic studies in two pigs to determine desirable plasma levels of orally administered tranilast, 36 crossbred juvenile pigs were randomized to placebo or tranilast before undergoing balloon angioplasty in both the left anterior descending and left circumflex plus stent implantation in the right coronary artery. Oral tranilast was administered at 3 g/day starting 3 days before coronary injury and continued for 28 days until euthanasia. Injured vessels were harvested and sections analyzed by computer-assisted microscopic planimetry.

RESULTS
In balloon-injured vessels, tranilast was associated with a 37% reduction in neointimal area normalized to fracture length (0.47 ± 0.01 vs. 0.74 ± 0.03 mm; p < 0.001) and a 23% reduction in adventital area normalized to vessel size (0.43 ± 0.02 vs. 0.56 ± 0.03; p = 0.003). In stented arteries, neointimal area normalized to injury score was 32% lower in the tranilast-treated group compared to control (1.94 ± 0.17 vs. 2.86 ± 0.29; p = 0.01).

CONCLUSIONS
In pig coronary arteries, tranilast was associated with a reduction in neointima formation and adventitial reaction after balloon injury. In stented vessels, tranilast was associated with a reduction in neointima formation normalized to injury score. (J Am Coll Cardiol 2000;35:1331–7) © 2000 by the American College of Cardiology

Restenosis remains the most problematic complication of percutaneous transluminal coronary angioplasty (PTCA) with significant costs to the health care system (1). Stent implantation initially appeared promising, with rates of 20% to 30% in so-called ideal lesions (2,3), but with improvement of stent technology, the indications of stenting have broadened, with a consequent restenosis rate ranging from 15% to 60% (4,5) at six months depending on lesion morphology and other factors. Pharmacological approaches in humans have been largely disappointing (6). However, recent clinical studies using oral tranilast have demonstrated promise through a decrease in the rates of angiographic restenosis (7).

Tranilast, N-(3,4-dimethoxycinnamoyl) anthranilic acid (molecular weight 327 Da), has been used for the prevention and treatment of keloids and hypertrophic scarring and for the treatment of a range of allergic conditions (8,9). In vitro studies using vascular smooth muscle cells (SMC), tranilast has been shown to inhibit cellular proliferation (10,11), cellular migration (10,11), collagen synthesis (10,11), angiotensin-II–mediated SMC contraction (12) and to restore cytokine-induced nitric oxide production against platelet-derived growth factor (PDGF) (13). Tranilast has also been shown to inhibit collagen synthesis in fibroblasts derived from hypertrophic tissues and keloids (14), to suppress mast cell degranulation and histamine release (15,16) and to suppress oxygen free radical production by neutrophils (17,18). In in vivo studies using either carotid or peripheral arteries of small animals (19–23), tranilast was effective in reducing neointimal area on histomorphometry.

Clinical efficacy studies evaluating tranilast for restenosis prevention have now been completed in over 700 Japanese
male patients who underwent PTCA. In the TREAT study (Tranilast REStenosis following Angioplasty Trial), a double-blind, randomized, multicenter trial, tranilast (600 mg/day for three months) reduced the angiographic restenosis rate at three months’ post-PTCA from 46.5% to 14.7%, a relative reduction of 68%, p < 0.001 (personal communication, Dr. H. Tamai). This result was confirmed by the TREAT-2 study, with similar relative reduction in the restenosis rate (personal communication, Dr. H. Tamai). Tranilast was also found to decrease the angiographic restenosis in stented lesions (personal communication, Dr. H. Tamai) and in patients undergoing directional coronary atherectomy (7). A large multicenter trial to investigate the effects of tranilast on restenosis (PRESTO) in over 11,000 patients undergoing PTCA and/or stent implantation has recently started.

Despite the clinical promise being shown by this oral agent for restenosis prevention, few data are available about the influence of tranilast on arterial wound healing in the setting of coronary interventions. We therefore investigated the effects of tranilast on histologic and histomorphometric changes in pig coronary arteries after balloon angioplasty and stent implantation.

METHODS

All experiments and animal care conformed to the National Institutes of Health and American Heart Association guidelines for the care and use of animals and were approved by the Emory University Institutional Animal Care and Use Committee.

Pharmacokinetic study. Because tranilast had not been previously used in pigs we examined the pharmacokinetic profile (E_max and t_1/2) of oral tranilast in juvenile pigs before initiating the efficacy study. A right atrial cannula was placed in two pigs via the jugular vein on the day prior to dosing. A first dose of either 50 to 100 mg/kg was given by mixing with corn syrup and pig chow; the alternate dose was given the following day. Blood samples (0, 30 min, 2, 8, and 24 h) were obtained following these single-dose oral administrations of tranilast. Plasma was separated by centrifugation, frozen, protected from light exposure and analyzed for tranilast content (Kissei Pharmaceutical, Japan). A dose of 100 mg/kg/day resulted in a peak plasma concentration of 38.6 ± 1.27 μg/ml (118 ± 3.9 μmol/liter) at 2 h and a t_1/2 of 8.9 h. Based on these findings and considering previous in vitro and in vivo animal studies (10–23), tranilast was given in the efficacy study at a dose of 1500 mg/pig twice daily. As pigs initially weighed between 22.8 and 31.3 kg, this corresponded to a dosing range of 95.8 to 131.6 mg/kg.

Efficacy study design. Thirty-six female or castrated male juvenile pigs (25 to 30 kg) were randomized to receive placebo (n = 18) or oral tranilast (n = 18). All pigs underwent balloon overstretch injury of the left anterior descending (LAD) and circumflex (CX) coronary arteries, and oversize stent implantation in the right coronary artery (RCA) according to established methods (24,25). Briefly, pigs were sedated with telazol (5 mg/kg IM), intubated and ventilated with isoflurane in a mixture of 50% O2 and 50% N2O at a rate of ~30 ml/kg/min. An 8F sheath and guiding catheter were introduced via the right femoral artery. Acetylsalicylate (2 mg/kg), heparin (200 IU/kg), and lidocaine (2 mg/kg) were administered intra-arterially. Angioplasty was performed using a 3.5-mm balloon, and a 4.0-mm balloon expandable stainless steel stent (slotted tube stainless steel PS153; Cordis, Miami Lakes, Florida) was implanted in the RCA after previous overstretch injury to achieve a 1:1.2–1.3 balloon-to-artery ratio; the operator was unaware of the treatment group assignment. Intracoronary nitroglycerin (200 μg) was administered in each artery before injury. Immediately postprocedure, angiograms were performed to assess vessel patency; the femoral sheath was removed, the femoral artery ligated, the skin closed and the animal allowed to recover. Pigs were returned to routine care including daily monitoring and drug feeding until 28 days.

Tissue processing and histomorphometry. Animals were euthanized with a lethal dose of pentobarbital, the heart was quickly removed and the coronary vasculature was perfusion-fixed with 10% buffered formalin at ~100 mm Hg pressure for 15 min. The heart was then immersed in 10% buffered formalin overnight. The balloon-injured arterial segments were cut into 3–4-mm segments, embedded in paraffin, sectioned to 4 μm thickness, and stained with hematoxylin–eosin (H&E), Verhoeff–van Gieson’s (VVG) elastic tissue stain and Masson’s trichrome. Injured arteries were defined as those in which there was a complete rupture of the tunica media. Stented arteries were embedded in methyl-butyl methacrylate and sectioned using a low-speed saw. Serial sections spanning the injury site were glued to acrylic slides, ground to ~100 μm thickness, polished to optical smoothness, and stained with H&E or VVG.

Abbreviations and Acronyms

AA = adventitial area
FL = fracture length
IA = neointimal area
IS = injury score
MIT = maximal intimal thickness
PDGF = platelet-derived growth factor
PTCA = percutaneous transluminal coronary angioplasty
SMC = smooth muscle cell
TGF = transforming growth factor
VA = vessel area

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Statistical analysis. The initial pharmacokinetic studies revealed that peak plasma concentrations were obtained at 2 h and the $t_{1/2}$ was found to be 8.9 h. Based on these findings, tranilast was given in the efficacy study at a dose of 1500 mg/pig twice daily, resulting in a dose range of 95.8 to 131.6 mg/kg in the study. Plasma levels of tranilast in treated pigs were $1.01 \pm 0.49$ mmol/liter at the time of angioplasty and $0.99 \pm 0.53$ mmol/liter at tissue harvest ($p = NS$); these values are about 10-fold higher than those obtained with a single-dose administration, indicating an accumulation in serum and/or tissue compartments with repeated administration. Serum levels of total protein, albumin, creatinine, transaminase, total bilirubin and lactate dehydrogenase were within normal limits and did not differ between the control and tranilast-treated groups.

**RESULTS**

**Tranilast dosing.** The initial pharmacokinetic studies revealed that peak plasma concentrations were obtained at 2 h and the $t_{1/2}$ was found to be 8.9 h. Based on these findings, tranilast was given in the efficacy study at a dose of 1500 mg/pig twice daily, resulting in a dose range of 95.8 to 131.6 mg/kg in the study. Plasma levels of tranilast in treated pigs were $1.01 \pm 0.49$ mmol/liter at the time of angioplasty and $0.99 \pm 0.53$ mmol/liter at tissue harvest ($p = NS$); these values are about 10-fold higher than those obtained with a single-dose administration, indicating an accumulation in serum and/or tissue compartments with repeated administration. Serum levels of total protein, albumin, creatinine, transaminase, total bilirubin and lactate dehydrogenase were within normal limits and did not differ between the control and tranilast-treated groups.

**Histology and histomorphometry.** Thirty-six animals underwent interventions on 108 coronary arteries; 72 arteries were ballooned and 36 stented. There were no significant differences in body weight between the tranilast-treated and control groups either at angioplasty ($26.9 \pm 0.7$ vs. $26.7 \pm 0.7$ kg) or 28 days ($35.2 \pm 1.0$ vs. $33.6 \pm 0.9$ kg).

**BALLOON-INJURED VESSELS.** Balloon injury was associated with complete medial rupture in 71 out of the 72 balloon-injured arteries, obvious neointima formation and an increase in AA, especially adjacent to the area of medial rupture (Fig. 1). Masson’s trichrome staining revealed abundant collagen in the neointima as well as adjacent adventitia. The histomorphometric changes due to balloon injury in the placebo and tranilast-treated groups are shown in Table 1. No significant differences existed between the treatment and control groups in either the extent of injury (as seen by the magnitudes of the FL) or the vessel size (as seen by the magnitudes of the VA).

**STENTED VESSELS.** All stents were seen to be well developed within the vessel, resulting in thinning of the media adjacent to the stent struts (Fig. 2). In the rare vessels with stent protrusion into the adventitia, there was evidence of perivascular hemorrhage. Inflammatory infiltrates adjacent to the stent struts were observed but with no discernible differences between tranilast-treated and control groups. The histomorphometry of the stented vessels is shown in Table 2. No significant difference was seen in vessel size (EELA) between tranilast-treated group and placebo. The IS ranged between 1 and 2.4 overall and was 1.60 $\pm 0.07$ for the treated group compared to 1.43 $\pm 0.08$ for the controls ($p = 0.14$). The neointimal area (IA) normalized to injury score (IA/IS) was significantly lower in the tranilast-treated group compared to controls ($1.94 \pm 0.14$ vs. $2.86 \pm 0.29$; $p = 0.01$). There was a strong trend toward reduction in absolute IA in the tranilast-treated group compared to controls ($3.13 \pm 0.30$ vs. $4.11 \pm 0.45$, $p = 0.07$). There was also a significant difference in the media area between the tranilast-treated group and placebo ($p = 0.05$) and a significant difference in IA/MA between the two groups ($1.16 \pm 0.14$ vs. $1.73 \pm 0.13$, $p = 0.002$).

**DISCUSSION**

The major findings of this study are that tranilast significantly reduced neointima formation as well as adventitial reaction after balloon injury at four weeks in pig coronary
arteries; in stented vessels, tranilast significantly reduced neointima formation normalized to IS. To our knowledge, this is the first study to demonstrate these effects. These data as well as preliminary clinical trials (7) suggest the potential for using tranilast as a clinical therapeutic strategy in coronary restenosis prevention.

In balloon-injured vessels, the significant reduction observed in neointima formation, coupled with the lack

**Figure 1.** Light microscopy of VVG-stained sections of pig coronary arteries harvested four weeks after overstretch balloon angioplasty. Top panels are from control; bottom panels from tranilast-treated animal. Left panels are 40× instrument magnification; right panels 200×. Note decreased neointima formation in artery of tranilast-treated pig despite balloon injury similar to control. L = lumen; M = tunica media; A = tunica adventitia; N = neointima.

**Table 1.** The Effect of Tranilast on the Vessel Response to Balloon Injury

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Tranilast Group</th>
<th>P Value</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA (mm²)</td>
<td>2.10 ± 0.11</td>
<td>2.23 ± 0.12</td>
<td>0.37</td>
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<tr>
<td>VA (mm²)</td>
<td>4.33 ± 0.19</td>
<td>3.96 ± 0.16</td>
<td>0.37</td>
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<tr>
<td>FL (mm)</td>
<td>1.75 ± 0.10</td>
<td>1.65 ± 0.10</td>
<td>0.29</td>
<td>5.7</td>
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<tr>
<td>AA (mm²)</td>
<td>6.68 ± 0.26</td>
<td>5.61 ± 0.21</td>
<td>0.004</td>
<td>16.0</td>
</tr>
<tr>
<td>MIT (mm)</td>
<td>0.63 ± 0.03</td>
<td>0.51 ± 0.02</td>
<td>0.009</td>
<td>19.0</td>
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<tr>
<td>LA/VA</td>
<td>0.48 ± 0.01</td>
<td>0.55 ± 0.01</td>
<td>&lt;0.001</td>
<td>−14.6</td>
</tr>
<tr>
<td>(AA-VA)/VA</td>
<td>0.56 ± 0.03</td>
<td>0.43 ± 0.02</td>
<td>0.003</td>
<td>23.2</td>
</tr>
<tr>
<td>IA (mm²)</td>
<td>1.32 ± 0.12</td>
<td>0.79 ± 0.06</td>
<td>&lt;0.001</td>
<td>40.2</td>
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<tr>
<td>MA (mm²)</td>
<td>0.91 ± 0.06</td>
<td>0.94 ± 0.04</td>
<td>0.71</td>
<td>−3.3</td>
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<tr>
<td>IA/FL (mm)</td>
<td>0.74 ± 0.03</td>
<td>0.47 ± 0.01</td>
<td>&lt;0.001</td>
<td>36.5</td>
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<tr>
<td>IA/MA</td>
<td>1.77 ± 0.24</td>
<td>0.88 ± 0.09</td>
<td>&lt;0.001</td>
<td>50.3</td>
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</table>

LA = luminal area; VA = vessel area; FL = fracture length; AA = adventitial area; MIT = maximal intimal thickness; IA = neointimal area; MA = medial area. Data are presented as mean value ± SEM.
of increase in the vessel area, suggests that tranilast may reduce restenosis by an inhibitory effect on neointima formation rather than by promoting chronic positive vessel remodeling. This lack of effect on vessel remodeling (i.e., alteration of EELA) occurred despite significant reduction in the adventitial reaction as determined by cross-sectional area, observed in the tranilast-treated group at four weeks.

In theory, the reduction in neointima formation due to tranilast could have resulted from an inhibition in cellular proliferation or a reduction in net extracellular matrix content. Several mechanisms of tranilast’s action that might

**Figure 2.** Light microscopy of H&E-stained sections of pig coronary arteries harvested four weeks after stenting. Top panels are from control; bottom panels from tranilast-treated animal. Left panels are 20× instrument magnification; right panels 200×. Note similar neointima formation in artery of tranilast-treated pig despite stent injury greater than control (arrows in panel 2c). L = lumen; M = tunica media; A = tunica adventitia; N = neointima.

**Table 2. The Effect of Tranilast on the Vessel Response to Stent Injury**

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Tranilast Group</th>
<th>p Value</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA (mm²)</td>
<td>4.51 ± 0.41</td>
<td>5.49 ± 0.38</td>
<td>0.087</td>
<td>−21.7</td>
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<tr>
<td>IELA (mm²)</td>
<td>8.62 ± 0.54</td>
<td>8.62 ± 0.30</td>
<td>1.00</td>
<td>0</td>
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<tr>
<td>EELA (mm²)</td>
<td>11.03 ± 0.66</td>
<td>11.61 ± 0.32</td>
<td>0.86</td>
<td>−5.2</td>
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<tr>
<td>MIT (mm)</td>
<td>0.86 ± 0.09</td>
<td>0.71 ± 0.07</td>
<td>0.24</td>
<td>17.4</td>
</tr>
<tr>
<td>IA (mm²)</td>
<td>4.11 ± 0.45</td>
<td>3.13 ± 0.30</td>
<td>0.07</td>
<td>23.8</td>
</tr>
<tr>
<td>IS</td>
<td>1.43 ± 0.08</td>
<td>1.6 ± 0.07</td>
<td>0.14</td>
<td>−11.8</td>
</tr>
<tr>
<td>MA (mm²)</td>
<td>2.41 ± 0.22</td>
<td>2.99 ± 0.24</td>
<td>0.05</td>
<td>−24.0</td>
</tr>
<tr>
<td>IA/IS</td>
<td>2.86 ± 0.29</td>
<td>1.94 ± 0.17</td>
<td>0.01</td>
<td>32.2</td>
</tr>
<tr>
<td>IA/MA</td>
<td>1.73 ± 0.13</td>
<td>1.16 ± 0.14</td>
<td>0.002</td>
<td>32.9</td>
</tr>
</tbody>
</table>

LA = luminal area; IELA = internal elastic lamina area; EELA = external elastic lamina area; MIT = maximal intimal thickness; IA = neointimal area; IS = injury score; MA = medial area. Data are presented as mean value ± SEM.
Inhibition of Neointima by Tranilast

In-stent restenosis is a vexing problem in interventional cardiology. Stents have decreased the restenosis rate by preventing early elastic recoil and late negative arterial remodeling, but profound neointima formation is the main cause of restenosis. Although statistically significant, the effects of tranilast in the stented group compared to the balloon group were less pronounced. In the tranilast-treated group, IA/IS was reduced by 32%. It has been suggested that pharmacological approaches may not be as effective in reducing stent versus balloon-induced neointima formation (36). Part of the reasoning behind this is that the stent prosthesis induces a foreign-body response in which both inflammation and continued low-level cell proliferation play important roles. Additionally, the thrombus burden in stented arteries may be more substantial than in balloon angioplasty (37). Despite these concerns oral administration of tranilast was associated with substantial reduction of neointima in stented arteries.

There has been concern about the potential side effects of tranilast, including organ and systemic toxicity involving the kidneys and liver. In our study we examined serum markers of kidney and liver function and did not observe an adverse effect of tranilast. Furthermore, the tranilast-treated pigs did not demonstrate any clinical symptoms suggestive of adverse cardiopulmonary or gastrointestinal sequelae. Shioda (8) and Okuda et al. (9) studied the anti-allergic properties of tranilast in children and adults and found no serious side effects. In a study that demonstrated reduction of restenosis after coronary atherectomy, liver dysfunction or abdominal discomfort was present in 5 of 45 patients (7); the large multicenter investigation now underway (PRESTO) should help elucidate whether tranilast can be given safely and effectively for restenosis prevention. A final potential complication with the use of tranilast might be that, owing to its property to limit collagen synthesis and fibroblast proliferation, a severely limited fibrotic tissue reaction could compromise the adherence of the stent to the artery wall; however, our results indicate that, although neointima was reduced, a sufficient fibrotic response was present to support tissue incorporation of the stents, as documented by VVG-stained stent sections showing fibrous neointima enveloping the stent struts in all samples.

Study limitations. Our results on the effect of tranilast after balloon injury and stenting should be interpreted cautiously and regarded as preliminary as they only reflect an interim response at four weeks. Studies evaluating the effect of tranilast on histomorphometric changes in the vessel wall at later time points have not been undertaken. The obvious possibility of interspecies variability in the biological response precludes direct extrapolation to humans. Furthermore, although our study demonstrated reduction of neointima and neoadventitia by tranilast, the putative mechanisms of these effects are as yet unknown.

Conclusions. Oral administration of tranilast inhibited neointima formation and adventitial reaction in balloon-injured pig coronary arteries at four weeks. In stented coronary arteries tranilast was associated with a significant reduction in neointimal area normalized to the extent of injury, and it showed a strong trend in reducing absolute neointimal area. These results could explain the beneficial effects seen with the initial clinical studies evaluating tranilast in restenosis prevention.

Acknowledgments
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