

A Nonantibiotic Chemically Modified Tetracycline (CMT-3) Inhibits Intimal Thickening

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Recent research has shown that the tetracycline antibiotics are pluripotent drugs that inhibit the activity of matrix metalloproteinases (MMPs) and affect many cellular functions including proliferation, migration, and matrix remodeling. We have shown that doxycycline inhibits MMP activity and intimal thickening after injury of the rat carotid artery, however we do not know whether these effects are because of the antibiotic, anti-MMP, or other actions of doxycycline. Recently, chemically modified tetracyclines have been synthesized that lack antibiotic activity but retain anti-MMP activity (CMT-3), or lack both antibiotic and anti-MMP activity (CMT-5). In the current study we have assessed the effects of treatment with CMT-3 or CMT-5 on intimal thickening after balloon catheter injury of the rat carotid artery. Rats were treated by oral gavage with 15 mg/kg/day CMT-3 or CMT-5. CMT-3 significantly reduced smooth muscle cell (SMC) proliferation in both the medial and intimal layers of the injured rat carotid artery compared to CMT-5. Furthermore, CMT-3 inhibited SMC migration from the media to the intima by 86% at 4 days after injury. CMT-3 also decreased MMP-2 activity. Finally, we found that CMT-3 treatment resulted in a significant reduction in intimal cross-sectional area from $0.23 \pm 0.01 \text{ mm}^2$ in the CMT-5 control group to $0.19 \pm 0.01 \text{ mm}^2$. There was also a reduction in elastin and collagen accumulation within the intima. We conclude that CMT-3 attenuated intimal thickening after arterial injury by inhibiting SMC proliferation, migration and MMP activity, and accumulation of extracellular matrix. The inhibitory effects of CMT-3 were independent of the antibiotic properties, but were dependent on the anti-MMP activity of the tetracycline family. (*Am J Pathol* 2003, 163:1557–1566)

The tetracyclines function as antibiotics by inhibiting bacterial protein synthesis,¹ but recent research has shown that they are pluripotent drugs that affect many functions in mammalian cells. Tetracyclines are potent inhibitors of the matrix metalloproteinase (MMP) family of enzymes,² and they have been used to reduce tissue degradation in

periodontal disease³ and arthritis.⁴ Doxycycline, a tetracycline derivative, has been used experimentally to inhibit matrix degradation during abdominal aortic aneurysm formation,^{5–8} and recent clinical studies have investigated the use of doxycycline to limit aneurysm growth.^{9–13}

Tetracyclines also inhibit cell proliferation, cell migration, and synthesis of the extracellular matrix in a variety of cell types studied in culture.^{14–21} Smooth muscle cell (SMC) proliferation, migration, and matrix synthesis contribute to the neointimal thickening observed in atherosclerosis, restenosis, and vein graft disease. Recently we tested doxycycline using an *in vivo* model of balloon catheter injury to the rat carotid artery, and showed that doxycycline inhibited SMC proliferation and migration, which led to an attenuation of intimal thickening.²² Furthermore, Loftus and colleagues²³ have shown that treatment with doxycycline reduces intimal thickening in vein grafts placed in organ culture. Taken together, these studies suggest that tetracyclines may be useful in the treatment of intimal thickening. However, given the multiplicity of effects, we do not know whether the antibiotic, anti-MMP, or other actions of doxycycline were responsible for the inhibition of intimal growth.

In the current study we use two chemically modified derivatives of tetracycline CMT-3 and CMT-5. CMT-3 (COL-3) is produced by deletion of the dimethylamino group from carbon 4 in the A ring of tetracycline, which abolishes the antibiotic activity but not the anti-MMP activity of the molecule. Further modification by replacement of the carbon 11 carbonyl oxygen and the carbon 12 hydroxyl groups with nitrogen, abolishes the anti-MMP activity, giving rise to CMT-5 (COL-5), which is neither antibiotic nor anti-MMP.²⁴ Our purpose was to compare the effects of CMT-3 and CMT-5 on intimal thickening using the rat carotid artery injury model.

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Materials and Methods

Surgery

Animal experiments were performed according to the guidelines of the Canada Council on Animal Care. Male Sprague-Dawley rats (Charles River, Constant, Quebec, Canada) weighing 375 to 415 g were used. Rats were anesthetized by intraperitoneal injection of 4.6 mg/kg xylazine (Rompum; Bayer Inc., Etobicoke, Ontario, Canada) and 70 mg/kg ketamine (Ketaset; Ayerst Veterinary Laboratories, Guelph, Ontario, Canada). Balloon catheter denudation of the left common carotid artery was performed as described previously.²⁵ CMT-3 (6-demethyl-6-deoxy-4-dedimethylamino tetracycline) and CMT-5 (a pyrazole derivative) were provided by CollaGenex Pharmaceuticals Inc., Newton, PA. The CMTs were administered daily by oral gavage at a dose of 15 mg/kg/day, starting 24 hours before surgery. This dose was chosen based on previous studies that found it to be the minimum effective dose abolishing MMP activity in the rat aorta in aneurysm studies.⁶ The CMTs were suspended at a concentration of 20 mg/ml in a solution of 1% carboxymethylcellulose containing 0.02% methyl 4-hydroxybenzoate, 0.01% propyl 4-hydroxybenzoate, 0.5% ethanol and 1% *N*-methyl-2-pyrrolidone. The rats were sacrificed at various time points after injury, chosen as follows based on previous studies elucidating the kinetics of the injury response. SMC proliferation was measured in the media (2 and 7 days) and intima (7 days).²⁶ Migration of cells from media to the intima and MMP activity were measured at 4 days.²⁵ Development of the neointima and accumulation of matrix were assessed at 14 days after injury. To label cells entering S phase in the 2- and 7-day groups, the rats were injected subcutaneously with three doses of 10 mg of 5-bromo-2'-deoxyuridine (BrdU; Boehringer Mannheim Corp., Montreal, Quebec, Canada) at 17, 8, and 1 hour before sacrifice. Rats were killed by an intraperitoneal injection of T61 (Invert Canada Ltd., Whitby, Ontario, Canada), then the carotids were perfusion fixed for 4 minutes with 4% paraformaldehyde in phosphate-buffered saline at a pressure of 120 mmHg. Paraffin-embedded sections were cut at positions 1-cm and 2-cm downstream of the origin of the common carotid artery and used for morphometric analysis.

Histomorphometry and Matrix Staining

SMC replication rates in the media and intima were measured 2 and 7 days after injury by immunostaining carotid cross-sections for BrdU and determining the percentage of BrdU-labeled cells present as previously described.²⁷ SMC apoptosis rates in the media at 2 days after injury were measured by terminal dUTP nick-end labeling of cross-sections using the Apoptag kit (Intergen, Purchase, NY) according to manufacturer's directions, and counting the number of terminal dUTP nick-end labeling positive SMCs within the media of each arterial section. At the 4-day time point, a 1-cm length was excised from the middle of the common carotid artery and used for assessment of SMC migration into the intima as previ-

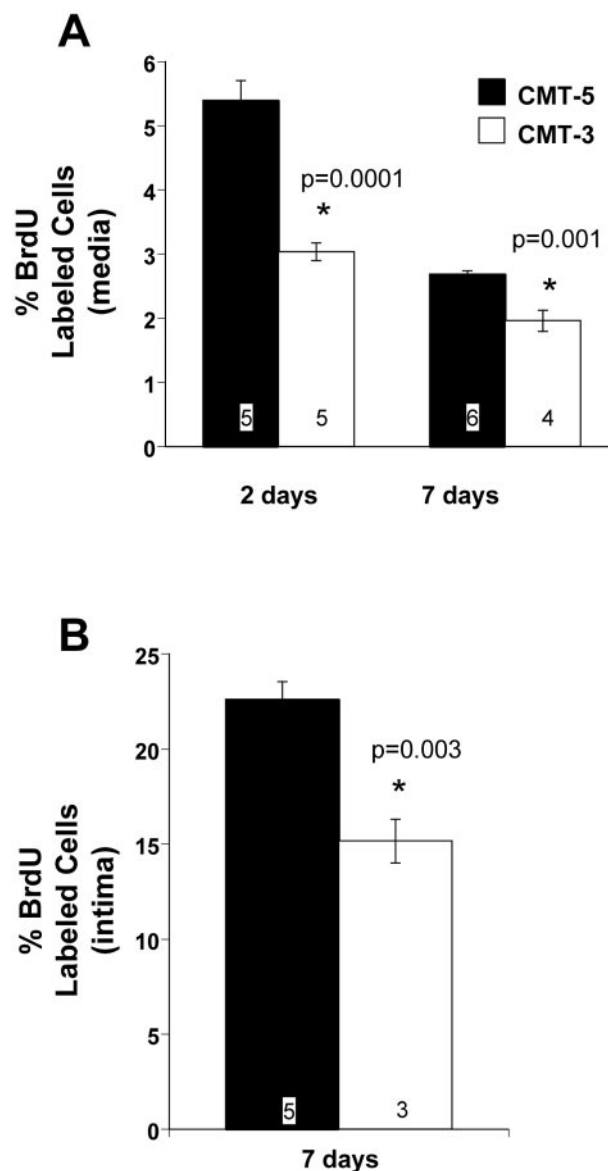


Figure 1. SMC replication in the media (**A**) and intima (**B**) after balloon catheter injury of the carotid artery. **Filled bars** represent values from CMT-5-treated control rats, and **open bars** values from CMT-3-treated rats. Values are mean \pm SEM; the number of rats in each group is indicated at the **bottom** of the bar. *, The value measured in the CMT-3 group was significantly less than the CMT-5 group.

ously described.²⁸ Briefly, intimal cells on the surface of the fixed common carotid artery segments were immunostained with an antibody against histone H1 (mAb 1276; Chemicon, Temecula, CA). The number of intimal cell nuclei per square mm of surface area was counted by light microscopy. The migration assay takes advantage of the fact that the first SMCs appear in the intima 3 to 4 days after injury; it takes ~24 hours for the cells to progress through the cell cycle, so the cells are counted before going through a round of replication.

Images of the cross-sections were obtained using a Nikon E600 microscope (Nikon, Mississauga, Ontario, Canada), digitized using a digital camera (model C4742-95-12NRB; Hamamatsu, Inc.) and analyzed using a computer-assisted morphometric analysis system (Simple; C

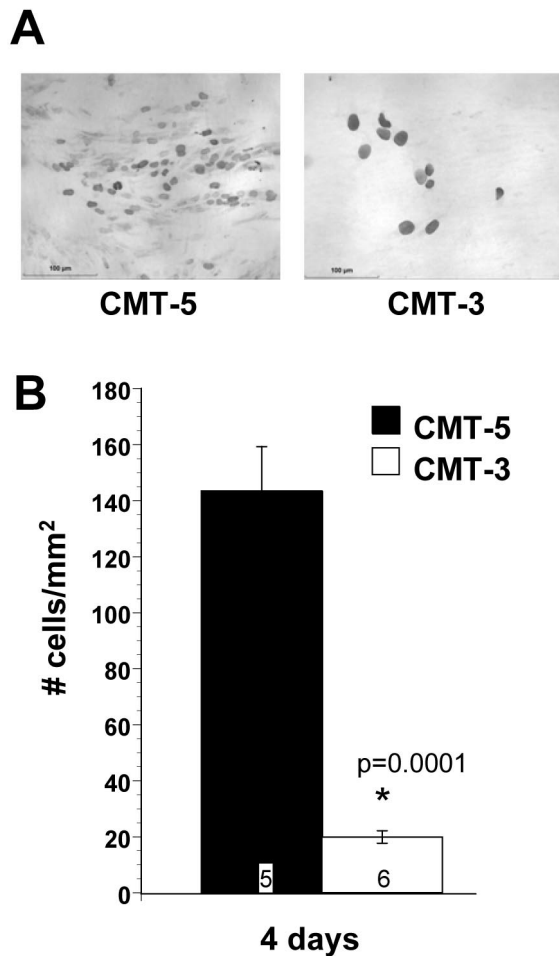


Figure 2. A: *En face* photomicrographs showing SMCs in the intima at 4 days after carotid injury. **B:** Quantitative analysis of SMC migration. **Filled bars** represent values from CMT-5-treated control rats, and **open bars** values from CMT-3-treated rats. Values are mean \pm SEM; the number of rats in each group is indicated at the **bottom** of the bar. *, The value measured in the CMT-3 group was significantly less than the CMT-5 group. Scale bar, 100 μ m (**A**).

Imaging Systems, Mars, PA). Measurements of intimal and medial areas (14 days after injury) were made as follows. Lumen area was determined by tracing around the inside edge of the vessel to determine the circumference, and calculating the lumen area assuming a circular geometry. Intimal area was measured as the area encompassed by the internal elastic lamina minus the lumen area (in this case lumen area was determined by tracing around the inside edge of the vessel and calculating the area inside). Medial area was measured as the area encompassed by the external elastic lamina minus the area encompassed by the internal elastic lamina (including lumen area). SMC density in the media and the intima was calculated by counting the total number of SMC nuclei within the layer on a cross-section, and dividing it by the cross-sectional area of that vessel layer.

Cross-sections taken 14 days after injury were also stained with Movat's pentachrome or picosirius red dye (PSR) to examine elastin and collagen within the matrix. To measure fibrillar collagen, an orientation-independent birefringence imaging system, LC-PolScope, (CRI, Woburn, MA) was used to perform quantitative polariza-

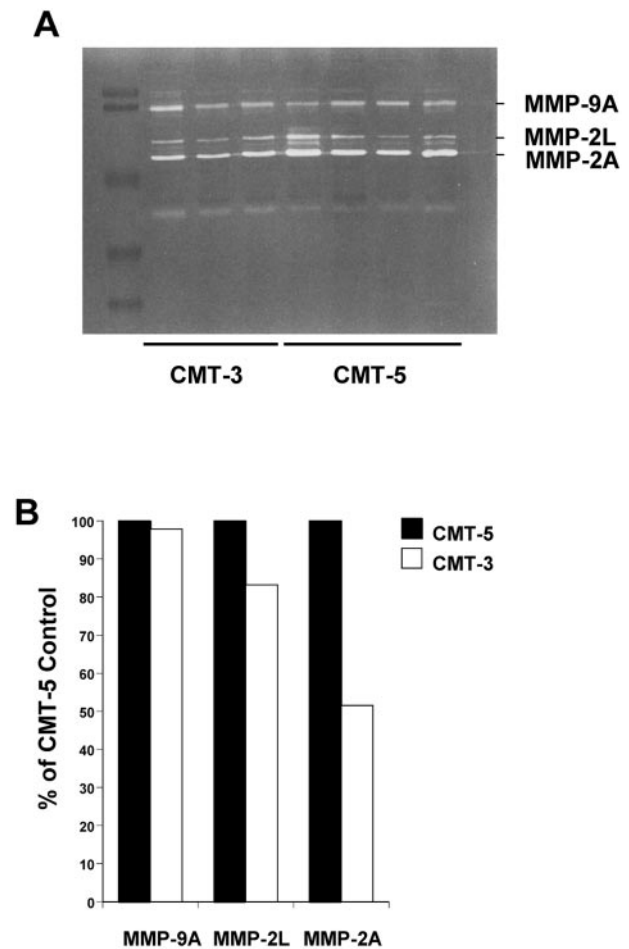


Figure 3. A: Gelatin zymogram showing the activity of MMP-9 active (88 kd) and MMP-2 (70-kd latent and 62-kd active) in carotid arteries from CMT-5- and CMT-3-treated rats 4 days after balloon catheter injury. Individual carotid extracts were prepared and 20 μ g of total protein from each was run in each well. **B:** Scanning densitometry was performed on zymogram gels, and the values for each MMP band were expressed as a percentage of the value obtained for the CMT-5 group. Four samples were averaged for the CMT-5 group and three for the CMT-3 group to obtain these measurements. **Filled bars** represent values from CMT-5-treated control rats, and **open bars** values from CMT-3-treated rats.

tion microscopy on PSR-stained sections.^{29,30} The system consists of liquid crystal filters, a polarization algorithm and a digital image processor. In PolScope images the brightness of each pixel is proportional to the birefringence retardance of the object. Using these images, specimen anisotropy (retardance and azimuth) were determined at all points simultaneously permitting measurements of the orientation and degree of organization of collagen fibers. Four rats per group were used for this analysis.

Zymograms

Seven rats were used to measure the activity of MMP-2 and MMP-9 in the carotid artery as previously described.²⁵ Extracts of individual carotid arteries were prepared, total protein content for each carotid was measured, and samples each containing 20 μ g of total protein were subject to electrophoresis on sodium dode-

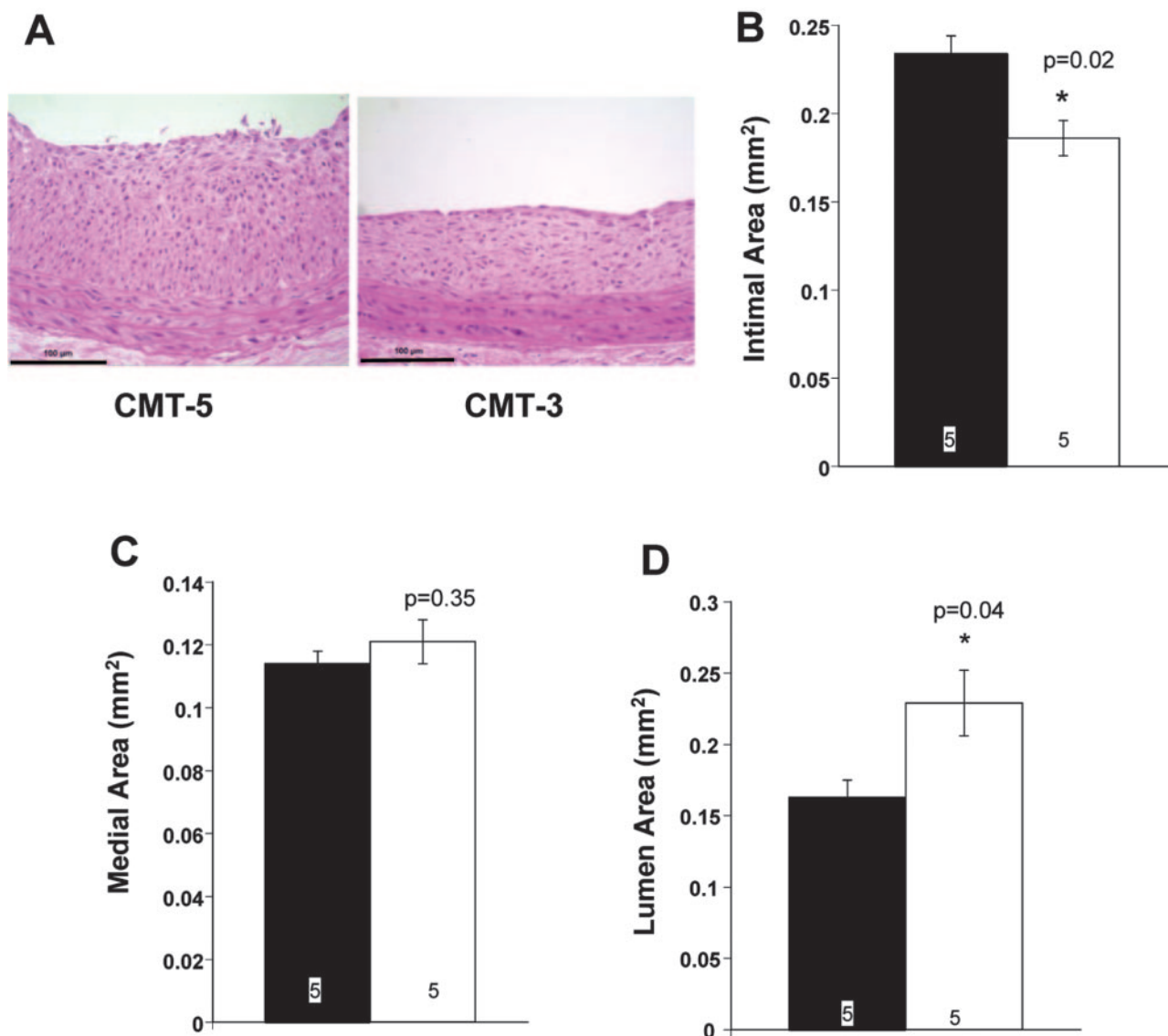


Figure 4. **A:** Photomicrographs of carotid cross-sections taken 14 days after balloon injury. Cross-sectional areas of the intima (**B**), media (**C**), and lumen (**D**) in carotid arteries from CMT-5 (filled bars)- and CMT-3 (open bars)-treated rats measured at 14 days after injury. Values are mean \pm SEM; the number of rats in each group is indicated at the bottom of the bar. *, The value measured in the CMT-3 group was significantly different from the CMT-5 group. Scale bar, 100 μ m (**A**).

cyl sulfate-polyacrylamide gel electrophoresis gels that contained 0.1% gelatin as a substrate for MMP digestion. After electrophoresis, the gels were incubated 16 hours, then stained with Coomassie Blue, and MMP activity was evident as cleared bands of substrate lysis. The MMPs were identified by their molecular weights and inhibition by ethylenediaminetetraacetic acid or phenanthroline. Activity was quantitated by scanning densitometric analysis using Scion Imaging Software (Scion Corp., Frederick, MD)

Statistical Analysis

Values are expressed as mean \pm SEM. Group means for CMT-3 versus CMT-5 treatment were compared by the two-tailed Student's *t*-test for independent samples.

Results

There were no significant differences in body weight between CMT-3- and CMT-5-treated rats.

SMC Proliferation

Previous studies have shown that medial SMC replication peaks at 2 days after balloon injury of the rat carotid artery, whereas intimal SMC replication peaks at 7 days. We found that medial SMC replication was significantly reduced by CMT-3 treatment at 2 and 7 days after injury, compared to CMT-5-treated rats (Figure 1A). At 2 days after injury, medial proliferation in the CMT-5-treated rats was $5.40 \pm 0.31\%$ compared to $3.04 \pm 0.14\%$ in the CMT-3-treated rats. At 7 days after arterial injury, medial SMC proliferation was reduced from $2.70 \pm 0.05\%$ in

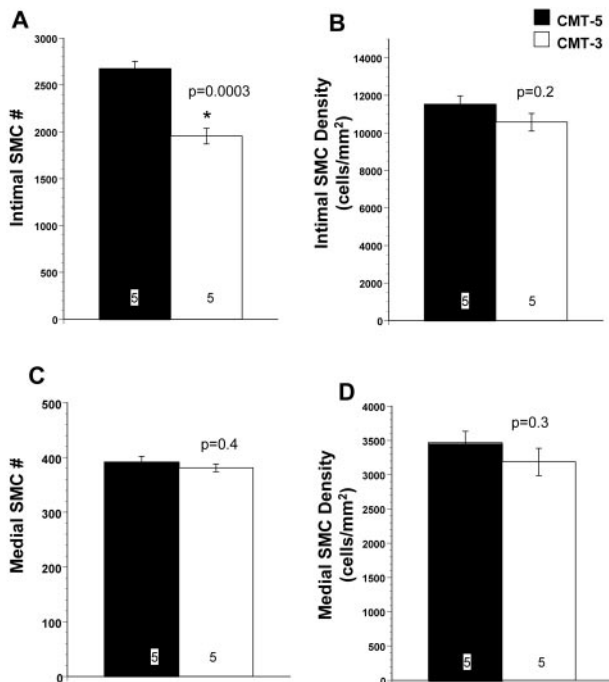


Figure 5. SMC number in the intima (A) and media (C), and SMC density per unit area in the intima (B) and media (D). Filled bars represent values from CMT-5-treated control rats, and open bars values from CMT-3-treated rats. Values are mean \pm SEM; the number of rats in each group is indicated at the bottom of the bar. *, The value measured in the CMT-3 group was significantly less than the CMT-5 group.

CMT-5-treated rats, to $1.96 \pm 0.67\%$ in the CMT-3-treated group. We measured intimal SMC replication at 7 days after balloon injury. CMT-3 treatment reduced intimal SMC proliferation from $22.6 \pm 0.9\%$ to $15.2 \pm 1.1\%$ at 7 days of balloon catheter injury (Figure 1B). There was no significant difference in the percentage of apoptotic SMCs in the media between the CMT-3- and the CMT-5-treated rats at 2 days after injury (data not shown).

SMC Migration

SMC migration from the media to the intima was measured at 4 days after injury. The first SMCs appeared in the intima 3 to 4 days after balloon injury, and it takes ~ 24 hours for the SMCs to complete a cycle of cell division, therefore this assay measured migration before the cells were able to proliferate within the intima. There were far fewer cells on the intimal surface of CMT-3-treated rats compared to CMT-5-treated rats at 4 days after injury (Figure 2A). The number of intimal SMCs was 143 ± 16 cells/mm² in CMT-5-treated rats compared to 20 ± 2 cells/mm² in CMT-3-treated rats (Figure 2B).

MMP Activity

Gelatin zymograms were used to assess MMP activity in the balloon-injured rat carotids. MMPs are secreted in a latent zymogen form, but the zymogen appears active on gels because sodium dodecyl sulfate present in the gel partially denatures the MMP exposing the active site.

Four major bands with molecular weights of 88, 70, 68, and 62 kd were visible on zymograms containing carotid extracts from CMT-5-treated rats (Figure 3A). We have previously shown that these lytic bands correspond to active MMP-9 (88 kd), the zymogen form of MMP-2 (70 kd), and active MMP-2 (62 kd), respectively.²⁵ The 68-kd band probably represents an intermediate activation product of MMP-2. In the arterial extracts from the CMT-3-treated rats, the intensity of all the MMP-2 bands was decreased compared to the CMT-5-treated rats (control) (Figure 3A). Densitometric analysis of the zymogram confirmed that in the CMT-3-treated group MMP-2 zymogen was reduced to 83%, and MMP-2 active was reduced to 52% of values in the CMT-5 group (Figure 3B). MMP-9 active was not affected by CMT-3 treatment.

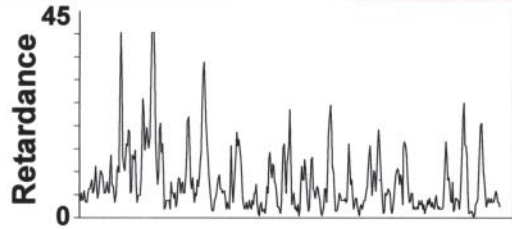
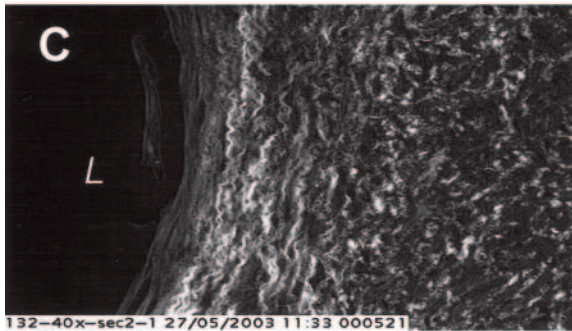
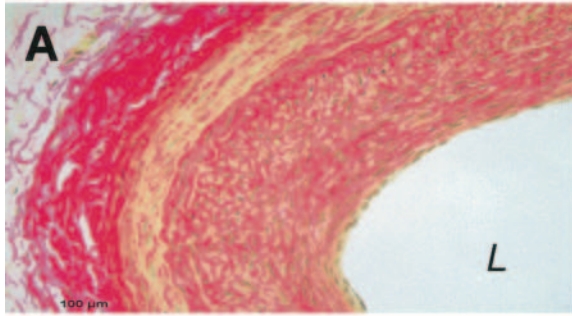
Vessel Wall Morphometry

Treatment with CMT-3 for 14 days after balloon catheter injury resulted in a marked decrease in intimal thickening when compared to the CMT-5-treated animals (Figure 4A). Intimal area measured on vessel cross-sections was 0.23 ± 0.01 mm² in the CMT-5 group compared to 0.19 ± 0.01 mm² in the CMT-3 group (Figure 4B). By contrast, there was no significant change in medial area between the two groups (Figure 4C). Lumen area was increased in the CMT-3 group; lumen area measured 0.23 ± 0.02 mm² in the CMT-3 group versus 0.16 ± 0.01 mm² in the CMT-5 group (Figure 4D). Two weeks after injury, intimal SMC number was significantly decreased from 2673 ± 80 in CMT-5-treated rats to 1954 ± 83 SMCs in CMT-3-treated rats (Figure 5A). Intimal SMC density was not different between the CMT-3 and CMT-5 groups (Figure 5B). Medial SMC number was not different between the two groups, nor was medial SMC density (Figure 5, C and D). There were no significant differences in internal elastic lamina or external elastic lamina perimeter between the two groups (data not shown) indicating that CMT-3 treatment did not alter the remodeling of vessel diameter.

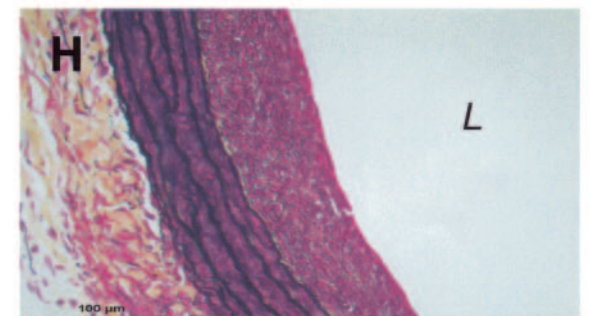
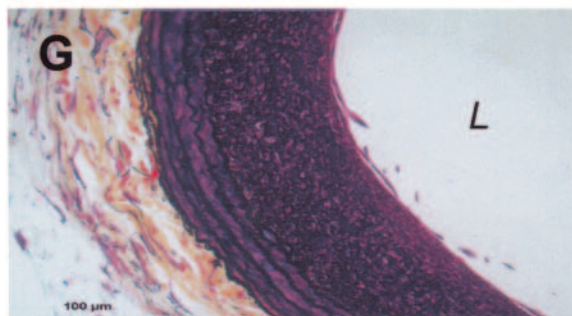
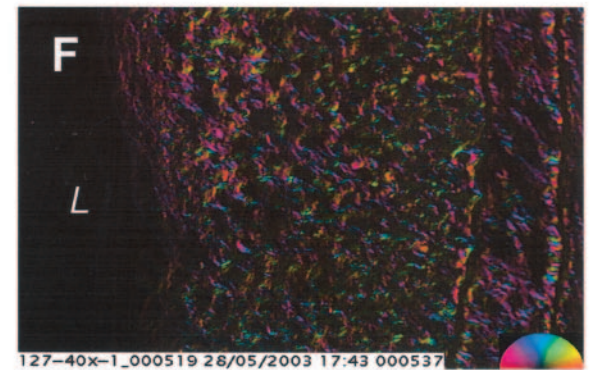
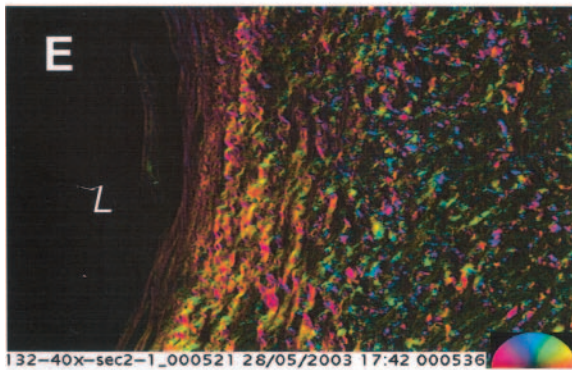
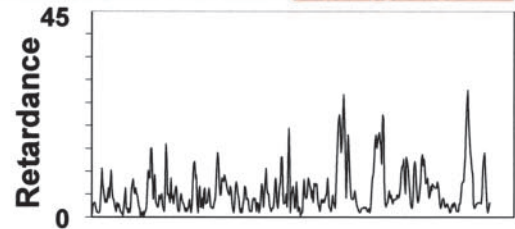
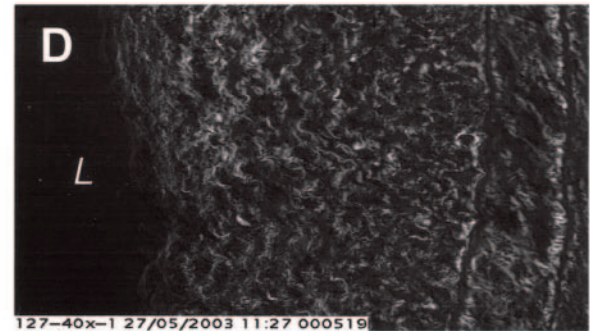
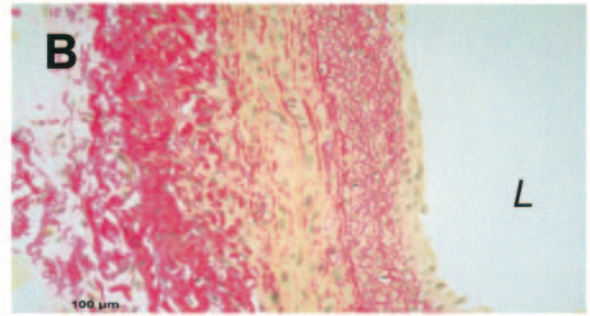
Collagen and Elastin Accumulation

Vessel cross-sections stained with PSR for collagen were visualized using transmitted light microscopy to determine the amount and the localization of collagen in the aortic wall (Figure 6, A and B). Decreased collagen staining was evident in the carotids from CMT-3-treated rats, especially in the intimal layer. The sections were also analyzed using quantitative polarized light microscopy to assess the content of collagen fibrils. PSR stains all collagen, however only collagen assembled into fibrils is birefringent and therefore visible by polarized light microscopy. Dense fibers of collagen were evident within the intimal layer of CMT-5 carotid arteries at 14 days after injury (Figure 6C). By contrast, there was less collagen apparent in the intima of CMT-3-treated rats (Figure 6D). Retardance values calculated across each section are shown underneath the photographs, and the results demonstrate abundant collagen fibers in the subluminal intima of the CMT-5-treated rats, but not the CMT-3 rats.

CMT-5



CMT-3



The PolScope was also used to assess fiber orientation, and this was measured and displayed on an azimuth map (Figure 6, E and F). This demonstrated a dense band of collagen fibers oriented in parallel within the innermost layer of the intima in the CMT-5-treated rats (Figure 6E), whereas the CMT-3-treated rats lacked this inner band of collagen fibers (Figure 6F). Average retardance values calculated from this inner layer of the intima, revealed a decrease in average retardance from 6.5 ± 1.9 in the CMT-5 group to 3.7 ± 0.8 in the CMT-3 group, indicating a decrease in collagen fiber content in the intima of the CMT-3-treated rats ($n = 4$ per group). A decrease in intimal elastin accumulation was also evident in the CMT-3-treated rats on visualization of sections stained with Movat's pentachrome [Figure 6, G (CMT-5) and H (CMT-3)].

Discussion

These experiments were performed to assess the effects of treatment with nonantibiotic derivatives of the tetracycline family on the arterial response to balloon catheter injury in the rat carotid. CMT-3 lacks antibiotic but retains anti-MMP activity, whereas CMT-5 lacks both antibiotic and anti-MMP activity. CMT-3 treatment significantly attenuated SMC proliferation and migration responses in the medial and intimal layers of the vessel wall, and reduced matrix accumulation in the intima, which ultimately resulted in the inhibition of intimal thickening. These results are in agreement with previous studies using doxycycline to inhibit intimal hyperplasia,^{22,23} and demonstrate that antibiotic activity is not necessary for the inhibition of intimal thickening.

It is well established that the MMPs play important roles in mediating intimal thickening after vascular injury.³¹ CMT-3 is a potent MMP inhibitor, and our observation of decreased MMP activity in the CMT-3-treated rats is in accord with other studies showing decreased MMP activity in vessels after doxycycline or CMT-3 treatment.^{5,6,9-11,22} The mechanisms by which the tetracyclines inhibit MMPs are not completely understood, but they are thought to act via both direct and indirect mechanisms. Tetracyclines bind to Zn^{2+} or Ca^{2+} associated with the MMP, blocking the active site or inducing conformational changes that render the proenzyme susceptible to fragmentation during activation.^{2,32} Although CMT-3 probably reduced MMP activity *in vivo* in our studies by this mechanism, the extent of this inhibition is probably underestimated by zymogram analysis, because CMT-3 is expected to dissociate away from the MMP during electrophoresis. The fact that we did observe a decrease in MMPs on the zymogram may indicate diminished MMP protein in the tissue samples. It is known that doxycycline and CMT-3 inhibit MMP produc-

tion by reducing the steady-state levels of mRNA for several of the MMPs.³³⁻³⁶ Our results show a reduction in the MMP-2 zymogen band on gelatin zymograms, suggesting that production of the enzyme may have been reduced, however further study will be required to determine whether there are decreases in mRNA and/or protein expression. We also saw decreases in the amount of active MMP-2 on the zymograms, therefore it is possible that CMT-3 prevented the *in vivo* activation of pro-MMP-2 zymogen by the MT1-MMP,³⁷ or by reactive oxygen species.³⁸ Another possibility is that CMT-3 inhibited inducible nitric oxide synthase activity,³⁹⁻⁴¹ thus reducing the production of nitric oxide, which is known to activate MMPs.⁴²

CMT-3 treatment dramatically inhibited SMC migration from the media to the intima. This is consistent with studies that demonstrate that the systemic administration of other MMP inhibitors,⁴³⁻⁴⁷ or the overexpression of tissue inhibitors of metalloproteinases,⁴⁸⁻⁵² significantly reduces the migration of SMCs after arterial injury. Previous studies have shown that CMT-3 inhibited the migration of several types of tumor cells *in vitro*,^{17,18,53,54} and reduced the formation of metastases in mouse tumor models.^{17,54,55} Based on these promising experimental results in cell migration studies, CMT-3 has been used in a phase I clinical trial to treat patients with metastatic cancers.⁵⁶

In the current study, we found that treatment with CMT-3 inhibited not only migration, but also SMC replication after arterial injury. CMT-3 attenuated the proliferation of both medial and intimal SMCs, in contrast to doxycycline which we found inhibited only intimal SMC replication.²² CMT-3 may be a more potent cytostatic agent than doxycycline; it was effective at lower concentrations than doxycycline inhibiting the growth of prostate cancer cells.⁵⁴ The mechanisms behind the anti-proliferative effects of the tetracyclines are not completely clear. It has been suggested that CMT-3 and other MMP inhibitors limit tumor cell proliferation by preventing the cleavage of cadherins from the cell surface, therefore preventing the release of β -catenin from the cadherin cytoplasmic domain and the translocation of β -catenin to the nucleus.^{18,57} Recent studies including our own have implicated cadherin- β -catenin signaling in the regulation of SMC migration and proliferation after vascular injury *in vivo*,⁵⁸⁻⁶⁰ and we can speculate that in the present study CMT-3 treatment inhibited cadherin- β -catenin signaling for migration and proliferation. However, there may also be direct effects on the cells, because cells treated with CMT-3 become arrested in the G_0/G_1 phase of the cell cycle because of a change in mitochondrial membrane potential.²⁰ CMT-3 has been shown to induce cell death by apoptosis,⁵⁴ however in the current study we observed no significant differences in the medial SMC ap-

Figure 6. Photomicrographs of carotid cross-sections taken 14 days after injury from CMT-5 (A, C, E, G) or CMT-3 (B, D, F, H)-treated rats. A and B: Sections stained with PSR for collagen visualized by transmitted light microscopy. C and D: PSR-stained sections visualized under polarized light microscopy and analyzed with a PolScope to show retardance because of birefringent collagen fibers. Retardance values calculated across the section are shown underneath. E and F: Pseudocolor images (azimuth map) representing birefringence slow axis orientation (ie, collagen fiber orientation). Color-coding scheme is seen at bottom right of each image, and color represents collagen orientation, while intensity represents magnitude. G and H: Sections stained with Movat's pentachrome and visualized by transmitted light microscopy to show elastin. Position of vessel lumen is indicated by L. Scale bars, 100 μ m.

optotic rate between the CMT-3- and CMT-5-treated groups.

The decreases in cell proliferation and migration that we observed after CMT-3 treatment contributed to a significant attenuation of intimal thickening after balloon injury. The decrease in intimal thickening after CMT-3 treatment was at least as great as we observed in a previous study using doxycycline.²² These results are also in agreement with those of Loftus and colleagues,²³ who showed that doxycycline inhibited intimal thickening in cultured human saphenous vein grafts. In addition, we found that CMT-3 treatment inhibited the accumulation of elastin and collagen in the injured intima, and the organization of collagen into dense parallel arrays of fibers adjacent to the lumen. Previous studies have demonstrated that the tetracycline antibiotics inhibit matrix synthesis,^{19,21} and we found that doxycycline inhibited elastin but not collagen accumulation in the injured rat carotid.²² Our current results suggest that CMT-3 inhibits both elastin and collagen accumulation, contributing to the reduction in intimal thickening.

One limitation of the rat carotid balloon injury model is that lumen loss is mainly because of neointimal hyperplasia, whereas in human restenosis, lumen loss is because of a combination of intimal hyperplasia and constrictive vessel remodeling. Several recent studies in porcine angioplasty models have implicated the MMPs in constrictive vessel remodeling,^{61–63} however, in another study administration of an MMP inhibitor failed to attenuate constrictive remodeling after angioplasty in monkeys.⁶⁴ We have shown that doxycycline inhibits constrictive remodeling of the rabbit abdominal aorta after balloon injury, secondary to inhibition of MMP activity and the inhibition of collagen fiber assembly in the vessel wall (Courtman and Bendeck, manuscript submitted). These data suggest that the tetracyclines are capable of inhibiting two critical mechanisms in restenosis, intimal hyperplasia and constrictive remodeling.

In conclusion, we showed that the nonantibiotic tetracycline derivative CMT-3 inhibited intimal hyperplasia after balloon injury of the rat carotid artery. Furthermore, CMT-3 was at least equivalent to, if not more effective than, doxycycline probably because of a greater anti-proliferative effect. This study demonstrates that the effects of the tetracyclines occur independent of antibiotic activity, seem to be dependant on anti-MMP activity, but may be accompanied by other nonantibiotic effects that could influence intimal thickening.

Acknowledgment

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