Development of a Water-Soluble Matrix Metalloproteinase Inhibitor as an Intra-arterial Infusion Drug for Prevention of Restenosis after Angioplasty

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To prevent restenosis after percutaneous transluminal coronary angioplasty (PTCA) and/or stenting of atherosclerotic stenosed arteries, we designed and developed two water-soluble matrix metalloproteinases (MMPs) inhibitors. The first inhibitor was monomeric in type and was chemically synthesized by succinylation of the synthetic MMP inhibitor, *N*-hydroxy-5-hydroxy-2(*S*)-methyl-4(*S*)-(4-phenoxybenzoyl)aminopentanamide (ONO-M11−335). The second inhibitor was polymeric and was a radical copolymer of the vinyl derivative of ONO-M11-335 and a water-soluble monomer, *N*,*N*-dimethylacrylamide (DMAAm). For the second inhibitor, NMR analyses and UV−vis spectra measurements showed that the content of the ONO-M11-335 unit in the copolymers (M_n; ca. 10 000 and 20 000 by GPC measurements) was about 8 per molecule. The MMP inhibitors were all highly soluble in water, even under neutral pH. The succinylated derivative markedly inhibited MMP-2, MMP-9, and MMP-12 in vitro, as did ONO-M11-335. In contrast the copolymers, which can maintain effective plasma levels for extended periods by prevention of hepatic uptake, showed a ca. 100-fold reduced inhibition activity. Such water-soluble MMP inhibitors, developed in this study, may potentially be useful as an intra-arterial infusion drug for vascular injury.

Introduction

Matrix metalloproteinases (MMPs) are classified into a series of the proteinase family and contain over 20 species of zinc-dependent endopeptidases.[1] The MMPs facilitate the degradation and remodeling of the extracellular matrix (ECM), including the interstitial and basement membranes, fibronectin, elastin, and laminin. The physiological role of the MMPs is to degrade ECM components, which promote connective tissue remodeling processes and which are expressed during the morphogenetic stage of embryonic development and differentiation, bone resorption, and tissue repair. The MMPs are also associated with various oncologic or pathological processes such as tumor invasion, rheumatoid joint destruction, uterine adenomyosis, and cardiovascular disease.[2]

In normal tissue, expression of MMP is regulated at the gene transcription level by extracellular stimulators[3] such as cytokines, growth factors, and cell or matrix−cell interactions. Once synthesized, they are secreted from cells as pre-proenzymes which can be activated by serine proteinases or other activated MMPs in the extracellular space.[4] In a defined activation mechanism, gelatinase A (MMP-2) is activated by interacting with a membrane type-1 MMP (MT1-MMP) that is activated preliminary by furin and by anchorage to the cell membrane surface.[5] The activity of MMP is inhibited by a natural tissue inhibitor of the metalloproteinase (TIMP), which is also secreted by transcriptional regulation and inhibits MMP activity by binding to the catalytic domain of the activated MMP.[6]

Percutaneous transluminal angioplasty in coronary and peripheral arteries (PTCA or PTA) has been widely used for clinical treatment of atheroscleotic stenosis using either balloon catheters or metallic stents. However, it has been reported that around 30−50% of restenosis cases have occurred few months after balloon angioplasty. Such a phenomenon remains an unsolved problem. One of the major causes of the restenosis was excessive tissue ingrowth (intimal hyperplasia), resulting from migration and proliferation of smooth muscle cells (SMC), and is triggered by degradation of the extracellular matrix by SMC derived MMP. MMP inhibitors therefore offer potential as a therapeutic tool for prevention of restenosis, and to this end, chemically synthesized MMP inhibitors have been widely developed over the past few decades.[7] X-ray crystallographic analyses of a single crystal of the MMP-inhibitor complex has been revealed together with determination of the enzyme's active site and the interaction mechanism between inhibitor and MMP.[8] Such information has been instrumental in the design of synthetic MMP inhibitors.[9] A number of MMP inhibitors, with varying efficiency, have been described which are involved in collagen peptidomimetics and nonpeptidomimetics.[10] These have been further classified according to the variety of their coordination groups binding to the zinc atom localized on the enzyme active site such as hydroxamate, phosphinate, thiol, and sulfodiimine. However, since most of these MMP inhibitors show extremely poor water solubility they are not maintained at high levels in the plasma, which is not suitable for an intra-arterial infusion drug.

In this study, we molecularly designed and developed two types of water-soluble MMP inhibitors by either derivatization of the carboxyl group or copolymerization

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Scheme 1. Synthetic Scheme of Water-Soluble Matrix Metalloproteinase Inhibitors

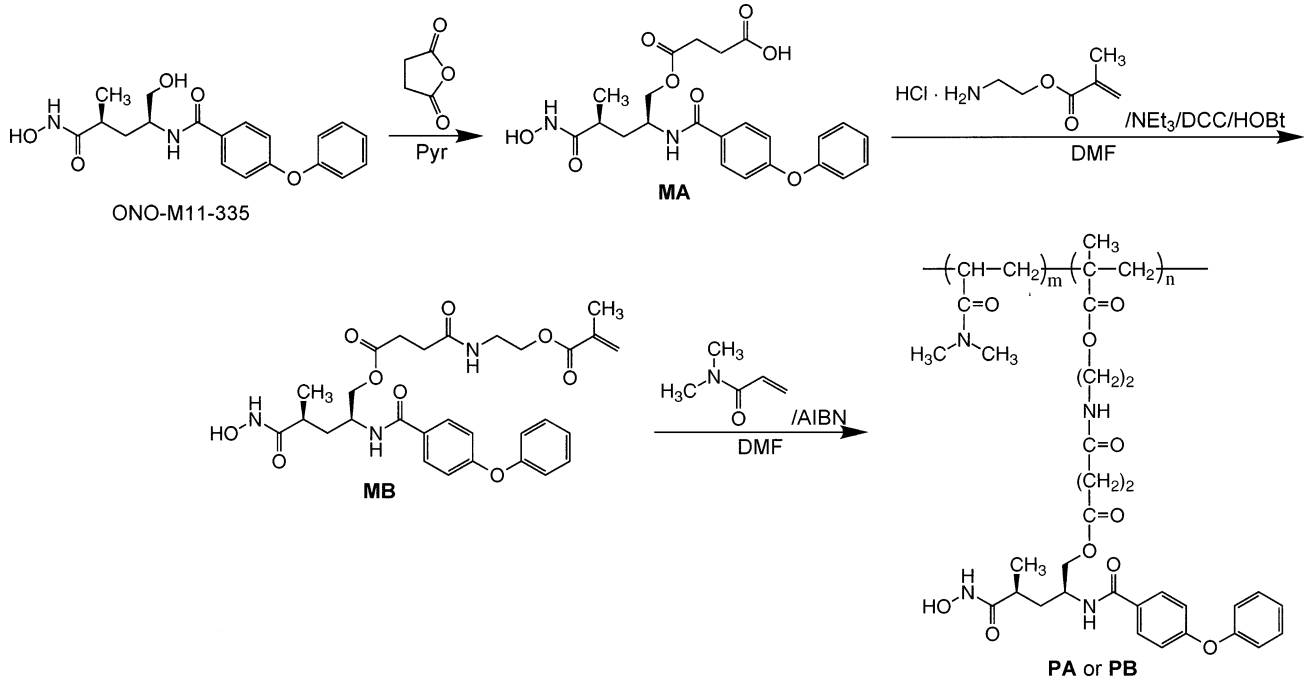

Table 1. Preparation of Poly(AEMA-ONO-*co*-DMAAm)

	feed of MB (mol %)	yield (%)	degree of derivatization of MB unit (mol %)		M_n ($\times 10^4$)	M_w/M_n	no. of MB units[c]/molecule
			^{1}H NMR[a]	UV[b]			
PA	5	70	5.4	6.0	1.8	2.81	7.8
PB	10	81	9.8	9.1	1.1	3.73	7.4

[a] Calculated from integration value ratio of $-CH_3$ protons of DMAAm vs aromatic ring protons of AEMA-ONO. [b] All analytical samples are prepared as 0.2 mg/mL of EtOH solution and molarity of ONO-M11-335 contained in sample solution was estimated by the molar extinction coefficient of MB at 253 nm. The λ_{max} was not different from monomer and copolymers. [c] Calculated from ^{1}H NMR.

with a water-soluble monomer to enable water-solubility of the synthetic MMP inhibitor ONO-M11-335. As for the copolymers, controlled drug delivery by hydrolysis in vivo was predicted. The potential abilities of the MMP inhibitors for clinical applications in intra-vascular surgery including PTCA and stenting are discussed.

Results

Preparation of Water-Soluble MMP Inhibitors. Two different molecular designs were used for the water-solubilization of the synthetic MMP inhibitor ONO-M11-335 (Scheme 1). The first approach involved synthesis of a monomeric inhibitor (**MA**), whereas the second approach involved synthesis of a polymeric inhibitor (**PA** and **PB**).

The monomeric MMP inhibitor (**MA**) was synthesized by derivatization of a succinic acid moiety to the ONO-M11-335 through an ester bond. The esterification of ONO-M11-335 was performed by nucleophilic attack of the 5-OH group of ONO-M11-335 to the carbonyl group of succinic anhydride in anhydrous pyridine at room temperature. The unfavorable nucleophilic attack of the nonprotected OH group of the hydroxamate moiety was preferentially prevented to minimize production of an amide derivative being negative to hydroxamic acid method.

The polymeric inhibitors (**PA** and **PB**) were synthesized by copolymerization of DMAAm with a vinyl derivative of ONO-M11-335 (**MB**) by an amide bond formation between **MA** and 2-aminoethyl methacrylate using DCC−HOBt as the coupling reagent. The amide bond procedure proceeded quantitatively with little side reactions or formation of byproducts resulting from the hydroxamate group. Thereafter, the ONO-M11-335 appending vinyl monomer (**MB**) was polymerized with DMAAm, in the presence of AIBN, as a conventional radical polymerization initiator (Scheme 1). Table 1 summarizes the composition of the obtained copolymers. The molecular weight of the copolymers (**PA** and **PB**) was around 20 000 and 10 000, respectively. The degree of derivatization of the **MB** unit in the copolymers was calculated using two alternative methods. The first method calculated the value from the ratio of the integrals of the aromatic ring protons ($\delta = 7.0-7.8$ ppm) of the MMP inhibitor unit with the dimethyl amide protons ($\delta = 2.6-3.4$ ppm) of the DMAAm unit in the ^{1}H NMR spectra. The second method determined the value from the UV/vis absorption spectrum of the copolymers using the absorption coefficient of the **MB**, which has a maximum absorption at a wavelength of 253 nm in ethanol ($\epsilon = 16\,230$). There was good agreement between the copolymer compositions calculated from ^{1}H NMR spectra and from UV/vis spectra. The degree of derivatization of MMP inhibitor unit in the copolymers was around 5 mol % (**PA**) and 10 mol % (**PB**), respectively. Thus, the number of the inhibitor unit in the two copolymers was about 8 per molecules. The succinylated derivative (**MA**) and two copolymers

Table 2. Solubility of MMP Inhibitors in Various Aqueous Solutions

	solubility (μg/mL)		
MMP inhibitor	artificial bile	buffer (pH 7.4)	purified water
ONO-M11-335	432	307	372
MA	>1000	>1000	398
PA	>1000	>1000	
PB	>1000	>1000	

Table 3. Human Matrix Metalloproteinase Inhibition Activities

	IC_{50} value (nM)[a]		
MMP inhibitor	MMP-2	MMP-9	MMP-12
ONO-4817	0.73	2.10	0.45
ONO-M11-335	0.65	0.80	3.50
MA	1.10	2.80	2.60
PA[b]	50	83	120
PB[b]	100	200	260

[a] 50% inhibitory concentration. [b] Exact molecular weights of polymers were not clear; however, their molar concentration was determined by a net inhibitor ratio involved in each polymers and number-average molecular weight estimated from GPC.

(**PA** and **PB**) were all very soluble in water even under neutral pH (Table 2).

Inhibition Activity for Human Matrix Metalloproteinase. ONO-M11-335 was a highly potent and relatively selective MMP inhibitor for MMP-2 and gelatinase B (MMP-9) compared with MMP-1 or MMP-7. The IC_{50} values (50% inhibitory concentration) for MMP-2, MMP-9, and MMP-12 (metalloelastase) were 0.65, 0.80, and 3.50 nM, respectively (Table 3). **MA** also showed high MMP inhibition activities comparable to ONO-M11-335, with IC_{50} values for MMP-2, MMP-9, and MMP-12 of 1.10, 2.80, and 2.60 nM, respectively. The IC_{50} values of MMP inhibitor-DMAAm copolymers for MMP-2, MMP-9, and MMP-12 were also estimated and were revealed to be 100, 200, and 260 nM for **PA** and 50, 83, and 120 nM for **PB**.

Discussion

Both vascular constructive remodeling and neointimal hyperplasia are major causes of restenosis in response to arterial injury after balloon angioplasty. In stenting, the possibility of constructive remodeling is almost excluded. It has, however, been reported that 20−30% of in-stent restenosis cases are observed a few months after stenting, which may be mainly due to neointimal hyperplasia, triggered by early SMC migration to the luminal surface of the artery after angioplasty injury and followed by SMC proliferation and ECM production. Therefore, one of the therapeutic strategies for the prevention of restenosis is the suppression of SMC migration using immunosuppressive agents such as FK506 and Rapamycin.[11] Both these agents bind to the cytosolic receptor. For instance, FK506 binds to FKBP12, which is physically associated with the type I TGF-β receptor and inhibits the SMC migration by blocking the growth factor mediated signal transduction pathways. Recent reports have shown that stents coated with these agents possessed sustained release from the polymer matrix and resulted in remarkably reduced in-stent restenosis.[12]

On the other hand, recent studies on the pathology of restenosis have focused on the role of ECM, which is concerned with structure and function, and the importance of the vascular ECM for the vascular reconstruction response to arterial injury, such as balloon and/or stent angioplasty. ECM degradation, which has a central role in SMC migration and proliferation during the early stages of neointimal hyperplasia, depends on a group of proteases known as MMPs. In mechanical arterial injury, overexpression of various MMPs, including stromelysin (MMP-3), collagenases (MMP-1), and gelatinases (MMP-2 and MMP-9) occurs during SMC migration.

Many different types of synthetic MMP inhibitors have been developed and assessed in clinical trials. For example marimastat,[13] a synthetic hydroxamate type MMP inhibitor, mimics the structure of collagen at the site where MMP binds to the zinc ion, which is located at the active site of the MMP and is held in a stereospecific manner. Marimastat is widely known to inhibit the activity of MMP-1, -2, -3, -7, and -9 but possesses a relatively nonspecific selectivity for the MMPs. Other peptide-mimetic hydroxamic acid type MMP inhibitors include GM 6001,[14] developed by Glycomed, Inc., which has been studied for its SMC antiproliferation activity in SMC migration assays and has been found to almost completely inhibit SMC migration, without reducing the number of cells available to migrate from the media to intima. On the other hand, ONO-4817,[15] a novel hydroxamic acid-based nonpeptide type orally active synthetic MMP inhibitor, was developed in ONO Pharmaceutical Co., which shows a broad inhibitory activity against MMPs except MMP-1 and MMP-7 at nanomolar concentration levels.

In contrast, our group developed a new percutaneous transluminal coronary angioplasty catheter with multiple functions for balloon inflation, local drug delivery, and coronary perfusion.[16−18] Water-soluble drugs can be infused from a port located distal to the inflated balloon during continuous blood perfusion via the perfusion lumen. Recently, local administration of several agents, including hepatocyte growth factor, docetaxel,[17] and a C-type natriuretic peptide,[18] directly to the injured site has attracted increasing interest as a potential therapeutic method for the prevention of restenosis.[19] Local drug delivery may allow a sufficient concentration of the drug to be achieved at tissue while minimizing systemic adverse effects. To use in intra-arterial infusion drug, water solubility is necessary. The synthetic works have been done for the design of water-soluble. For example, Scozzafava et al. synthesized water-soluble sulfonamides by attaching water-solubilizing moieties such as pyridine carboximide, carboxypyridinecarboxamide, etc., in which carbonic anhydrase inhibitors derivatized with water-soluble tail structure showed topical activity as an antiglaucoma drug.[20] In this study, we first designed two alternative approaches for water solubilization of the MMP inhibitor ONO-M11-335 by applying simple chemical reactions. As expected, both MMP inhibitors were highly soluble in water even under neutral pH (Table 2). The first inhibitor, which is monomeric succinate derivative (**MA**), can potentially acquire high doses at local areas using the above-mentioned multiple functional balloon catheter. On the other hand, the second inhibitor, which is copolymer with *N*,*N*-dimethylacrylamide (**PA** and **PB**), can potentially acquire an

effective plasma level for long periods by prevention of hepatic uptake in addition to the local administration activity.

The in vitro enzyme assay showed that the succinylated MMP inhibitor and the prodrug polymers maintained their inhibition activity for the human matrix metalloproteinases after chemical modification. Comparing the MMP inhibition activities with ONO-4817, which is only slightly soluble in water, the IC_{50} values for each of the MMPs were approximately identical. Due to the high water solubility of these water-soluble MMP inhibitors, they offer many advantages for drug dose dependent clinical trials and may suppress intimal hyperplasia when the dose concentration of the MMP inhibitors is increased. As for the prodrug polymers, since the inhibition activities showed relatively good retention they offer the potential for antirestenosis therapy. However, the IC_{50} values for the prodrug polymers indicated remarkably inferior inhibition activities against all the MMPs compared to the monomeric MMP inhibitors. Such inactivation is attributed to the steric hindrance of the MMP inhibitor moiety, which inhibits other MMP groups from binding to the enzyme's active site. **PA**, the lower **MB** contents copolymer, showed about twice as much activity as **PB**. However, copolymerization of non water-soluble synthetic MMP inhibitors, such as ONO-4817, with monomers of excellent water solubility, such as *N*,*N*-dimethylacrylamide, is an advantageous strategy to maintain drug concentrations at high levels in plasma and is expected to express prodrug-like controlled drug delivery functions by hydrolysis in vivo. Further in vitro studies of SMC migration assays using matrigel, in the presence of **MA**, **PA**, and **PB** are currently in progress, as are in vivo inhibition studies of experimental neointimal hyperplasia using local drug delivery systems with the infusion balloon catheter.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded in DMSO-d_6 or D_2O using tetramethylsilane (0 ppm) as an internal standard with a 270 MHz NMR spectrometer (JEOL, GX-270, Tokyo, Japan) at room temperature. UV−vis absorption spectra were measured on a Pharmaspec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). Gel permeation chromatography (GPC) analyses in *N*,*N*-dimethylformamide were carried out with a HPLC-8020 instrument (Tosoh, Tokyo, Japan) (column: Tosoh TSKgel α-3000 and α-5000). The columns were calibrated with narrow weight distribution poly(ethylene glycol) standards.

In Vitro Enzyme Assay. MMPs were extracted and purified from human normal dermal fibroblast. To an assayed buffer solution (40 μL) of the purified MMP (5.0 μM), *p*-aminophenylmercuric acetate (APMA: 5.0 μL, 10 mM) was added to activate the enzyme and preincubated at 37 °C for 1 h. A buffer solution (20 mL) of synthetic substrates, (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(*N*3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl)-Ala-Arg-NH_2 (McaPLDG-LDpaAR)[21] (130 μL; final concentration: 13.5 μM) with or without MMP inhibitor were incubated at 37 °C for 5 min prior to addition of the preincubated activate enzyme (50 μL/well) to each solution. The enzymatic assay was performed at 37 °C after 15 min incubation. The enzymatic activities were represented by an increase in the fluorescent intensity at 393 nm (excitation: 325 nm) per 1 min over the period of incubation. Inhibition activity, the IC_{50} values, was determined from plots of percentage inhibition vs log of inhibitor concentration.

Materials. ONO-M11-335 was kindly supplied from ONO Pharmaceutical Co., Ltd. (Osaka, Japan) and used as received. *N*-Hydroxybenztriazole (HOBt) was of special grade and purchased from Peptide Institute Inc. (Osaka, Japan) and used without further purification. 2-Aminoethyl methacrylate·HCl was purchased from Polyscience Inc., (Niles, IL) and other chemical reagents were commercially obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Anhydrous solvents were distilled after drying.

Synthesis: *N*-Hydroxy-5-carboxylethylcarbonyloxy-2(*S*)-methyl-4(*S*)-(4-phenoxybenzoyl)aminopentanamide (MA). To an anhydrous pyridine solution (5 mL) of ONO-M11-335 (500 mg, 1.4 mmol) was added dropwise at 0 °C an anhydrous pyridine solution (5 mL) of succinic anhydride (280 mg, 2.8 mmol), and the mixture was then stirred overnight at room temperature. The reaction mixture was diluted with AcOEt (150 mL) and washed with 1 N HCl (50 mL × 2) and then with 5% $NaHCO_3$ (50 mL × 2). The pH of the aqueous layer was adjusted to pH 5 with 1 N HCl and then extracted in AcOEt (50 mL × 2). After drying over $MgSO_4$, the solvent was removed by vacuum evaporation after filtration. The white solid was purified by silica gel column chromatography (eluent: chloroform/methanol, from 10/1 to 5/1) to give the desired product as a white solid: yield 180 mg (28%); FAB-MASS *m*/*n* 459.18 [M + H$^+$]; M_r = 458.47 calcd for $C_{23}H_{26}N_2O_8$; ^{1}H NMR (D_2O + Na_2CO_3) δ 0.98 (d, 3H, *J* = 6.9 Hz, −CH_3), 1.63−1.96 (m, 2H, −CH(CH_3)CH_2−), 2.21−2.28 (q, 1H, *J* = 7.2 Hz, −C*H*(CH_3)−), 2.30 (t, 2H, *J* = 6.0 Hz, −CH_2C$H_2$$CO_2$H), 2.45 (t, 2H, *J* = 6.0 Hz, −CH_2C$H_2$$CO_2$H), 4.02−4.28 (m, 3H, −C*H*CH_2O−), 6.96−7.05 (m, 4H, *m*-H of −COC_6H_4− and *o*-H of −OC_6H_5), 7.14 (t, 1H, *J* = 7.5 Hz, *p*-H of −OC_6H_5), 7.33 (t, 2H, *J* = 7.5 Hz, *m*-H of −OC_6H_5), 7.61 (d, 2H, *J* = 7.5 Hz, *o*-H of −COC_6H_4−). Anal. ($C_{23}H_{26}N_2O_8$) C, H, N.

***N*-Hydroxy-5-[2-[*N*-[2-(isopropenylcarbonyloxy)ethyl]-carbamoyl]ethylcarbonyloxy]-2(*S*)-methyl-4(*S*)-(4-phenoxybenzoyl)aminopentanamide (MB).** To an anhydrous DMF solution (5 mL) of **MA** (778 mg, 1.7 mmol), 2-aminomethacrylate·HCl (566 mg, 3.4 mmol), HOBt (344 mg, 2.55 mmol), and NEt_3 (523 μL, 3.74 mmol) was added dropwise at −5 °C DCC (421 mg, 2.0 mmol) in anhydrous DMF (5 mL), and the mixture was stirred for 2 h followed by stirring overnight at room temperature. The reaction mixture was extracted into ethyl acetate and washed successively with three 50 mL portions of 1 N HCl, three 50 mL portions of 5% $NaHCO_3$, and 50 mL of brine, followed the separation of the organic layer, drying over $MgSO_4$, condensation, and purification by chromatography on silica gel (eluent: chloroform/methanol = 15/1) to give the desired product as a white solid: yield 536 mg (55%); FAB-MASS *m*/*n* 570.25 [M + H$^+$]; M_r = 569.61 calcd for $C_{29}H_{35}N_3O_9$; ^{1}H NMR (DMSO-d_6) δ 1.04 (d, 3H, *J* = 5.4 Hz, −CH_3), 1.60−1.77 (m, 2H, −CH(CH_3)CH_2−), 1.87 (s, 3H, −C(CH_3)=CH_2), 2.23−2.48 (m, 5H, −OCOCH_2CH_2CONH− and −C*H*(CH_3)−), 3.31 (m, 2H, −CONHC$H_2$$CH_2$OCO−), 4.07−4.21 (m, 5H, −CONHCH_2CH_2OCO− and −C*H*CH_2O−), 5.60 and 6.01 (s, 2H, −C(CH_3)=CH_2), 6.99−7.06 (t, 4H, *J* = 8.1 Hz, *m*-H of −COC_6H_4− and *o*-H of −OC_6H_5), 7.17 (t, 1H, *J* = 8.1 Hz, *p*-H of −OC_6H_5), 7.43 (t, 2H, *J* = 8.1 Hz, *m*-H of −OC_6H_5), 7.82 (d, 2H, *J* = 8.1 Hz, *o*-H of −COC_6H_4−). Anal. ($C_{29}H_{35}N_3O_9$) C, H, N.

Poly[*N*,*N*-dimethylacrylamide-*co*-*N*-hydroxy-5-[2-[*N*-[2-(isopropenylcarbonyloxy)ethyl]carbamoyl]ethylcarbonyloxy]-2(*S*)-methyl-4(*S*)-(4-phenoxybenzoyl)aminopentanamide] (PA and PB). Two copolymers of **MB** and DMAAm were prepared by radical copolymerization. A typical procedure is as follows. A glass tube containing a mixture of **MB** (80 mg, mmol), DMAAm (250 mg, mmol), 2,2′-azobis(isobutyronitrile) (AIBN) (4.36 mg, mmol), and DMF (11 mL) was sealed under reduced pressure after three freeze−pump−thaw cycles. After shaking at 60 °C for 24 h, the precipitate, obtained by addition of ether, was separated from the solution by filtration. Reprecipitation was carried out in a DMF−ether system three times. The last precipitate was dried under vacuum. The yield of poly(AEMA-ONO-*co*-DMAAm) was 230 mg (70%). The molecular weight was estimated by GPC analysis: M_n =18 000; ^{1}H NMR (in D_2O) δ 0.96 (d, 24H, *J* = 5.4 Hz, −CH(CH_3)-), 1.24−1.54 (m, 352H, CH_2 and CH_3 of

main chain, $-CH(CH_3)CH_2-$), 2.12−2.14 (m, 8H, $-CH(CH_3)-$), 2.31−2.98 (m, 848H, $-N(CH_3)_2$ and $-OCOCH_2CH_2CONH-$), 3.43−3.58 (m, 24H, $-CONHCH_2CH_2OCO-$ and $-CHCH_2O-$), 4.00 (m, 16H, $-CHCH_2O-$), 6.95−7.03 (2d, 32H, J = 8.4 Hz, *m*-H of $-COC_6H_4-$ and *o*-H of $-OC_6H_5$), 7.13 (t, 8H, J = 8.4 Hz, *p*-H of $-OC_6H_5$), 7.33 (t, 16H, J = 8.4 Hz, *m*-H of $-OC_6H_5$), 7.63 (d, 16H, J = 8.4 Hz, *o*-H of $-COC_6H_4-$). The degree of derivatization of **MB** unit was 5 mol % by UV spectral measurement. Copolymer with 10 mol % of **MB** unit was synthesized in a similar procedure by changing the monomer ratio in the feed (data in the text).

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Supporting Information Available: ^{1}H NMR spectrum of **PA**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Massova, I.; Kotra, L. P.; Fridman, R.; Mobashery, S. Matrix metaloproteinases: structures, evolution, and diversification. *FASEB J.* **1998**, *12*, 1075−1095. Hidalgo, M.; Eckhardt, S. G. Development of matrix metalloproteinase inhibitors in cancer therapy. *J. Natl. Cancer Inst.* **2001**, *93*, 178−193.

(2) Dollery, C. M.; McEwan, J. R.; Henney, A. M. Matrix metalloproteinases and cardiovasucular disease. *Circ. Res.* **1995**, *77*, 863−868.

(3) Galis, Z. S.; Muszynski, M.; Sukhova, G. K.; Simon-Morrissey, E.; Unemori, E. N.; Lark, M. W.; Amento, E.; Libby, P. Cytokine-stimulated human vascular smooth muscle cells synthesized a complement of enzymes required for extracellular matrix digestion. *Circ. Res.* **1994**, *75*, 181−189.

(4) Nagase, H. Activation mechanisms of matrix metalloproteinases. *Biol. Chem.* **1997**, *378*, 151−160.

(5) Sato, H.; Takino, T.; Okada, Y.; Cao, J.; Shinagawa, A.; Yamamoto, E.; Seiki M. A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* **1994**, *370*, 61−65.

(6) Baker, A. H.; Zaltsman, A. B.; George, S. J.; Newby, A. C. Divergent effects of tissue inhibitor of metalloproteinase-1, -2 or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vivo. *J. Clin. Invest.* **1998**, *101*, 1478−1487.

(7) Supuran, C. T.; Scozzafava, A. In *Matrix metalloproteinases (MMPs) in proteinase and peptidase inhibition: Recent potential targets for drug development*; Smith, H. J., Simons, C., Eds.; Taylor & Francis: London & New York, 2002; pp 35−61. Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem. Rev.* **1999**, *99*, 2735−2776.

(8) Matter, H.; Schwab, W.; Barbier, D.; Billen, G.; Hasse, B.; Neises, B.; Schudok, M.; Thorwart, W.; Schreuder, H.; Brachvogel, V.; Lonze, P.; Weithmann, KU. Quantitative structure−activity relationship of human neutrophil collagenase (MMP-8) inhibitors using comparative molecular field analysis and X-ray structure analysis. *J. Med. Chem.* **1999**, *42*, 1908−1920.

(9) Gavuzzo, E.; Pochetti, G.; Mazza, F.; Gallina, C.; Gorini, B.; D'Alessio, S.; Pieper, M.; Tschesche, H.; Tucker, P. A. Two crystal structures of human neutrophil collagenase, one complexed with a prime- and the other with an unprimed-side inhibitor: implications for drug design. *J. Med. Chem.* **2000**, 43, 3377−3385.

(10) Jia, MC.; Schwartz, M. A.; Sang, Q. A. Suppression of human microvascular endothelial cell invasion and morphogenesis with synthetic matrixin inhibitors. Targeting angiogenesis with MMP inhibitors. *Adv. Exp. Med. Biol.* **2000**, *476*, 181−94. Graf von Roedern, E.; Grams, F.; Brandstetter, H.; Moroder, L. Design and synthesis of malonic acid-based inhibitors of human neutrophil collagenase (MMP8). *J. Med. Chem.* **1998**, *41*, 339−345.

(11) Poon, M.; Sun, J.; Marx, S. O.; Gallo, R.; Badimon, J. J.; Taubman, M. B.; Marks, A. R. Rapamycin inhibits vascular smooth muscle cell migration. *J. Clin. Invest.* **1996**, *98*, 2277−2283. Sun, J.; Marx, S. O.; Chen, H.-J.; Poon, M.; Marks, A. R.; Rabbani, L. E. Role for p27^{Kip1} in vascular muscle cell migration. *Circulation* **2001**, *103*, 2967−2972.

(12) Marx, S. O.; Marks, A. R. The development of Rapamycin and its application to stent restenosis. *Circulation* **2001**, *104*, 852−855.

(13) Sierevogel, M. J.; Pasterkamp, G.; Velema, E.; de Jaegere, P. P. T.; de Smet, B. J. G. L.; Verheijen, J. H.; de Kleijn, D. P. V.; Borst, C. Oral matrix metalloproteinase inhibition and arterial remodeling after angioplasty. *Circulation* **2001**, *103*, 302−27. Margolin, L.; Fishbein, I.; Banai, S.; Golomb, G.; Reich, R.; Perez, L. S.; Gertz, S. D. Metalloproteinase inhibitor attenuates neointima formation and constrictive remodeling after angioplasty in rats: augmentative effect of $\alpha_{\nu}\beta_3$ receptor blockade. *Atherosclerosis* **2002**, *163*, 269−277.

(14) Li, C.; Cantor, W. J.; Nili, N.; Robinson, R.; Fenkell, L.; Tran, Y. L.; Whittingham, H. A.; Tsui, W.; Cheema, A. N.; Sparkes, J. D.; Pritzker, K.; Levy, D. E.; Strauss, B. H. Arterial repair after stenting and the effects of GM6001, a matrix metalloproteinase inhibitor. *J. Am. Col. Card.* **2002**, *39*, 1852−1858.

(15) Yamada, A.; Uegaki, A.; Nakamura, T.; Ogawa, K. ONO-4817, an orally active matrix metalloproteinase inhibitor, prevents lipopolysaccharide-induced proteoglycan release from the joint cartilage in guinea pigs. *Inflamm. Res.* **2000**, 49, 144−146. Mori, T.; Yamasaki, S.; Masui, F.; Matsuda, M.; Sasabe, H.; Zhou, Y. F. Suppression of the development of experimentally induced uterine adenomyosis by a novel matrix metalloproteinase inhibitor, ONO-4817, in mice. *Exp. Biol. Med.* **2001**, *226*, 429−433. Muraishi, Y.; Mitani, N.; Fuse, H.; Saiki, I. Effect of a matrix metalloproteinase inhibitor (ONO-4817) on lung metastasis of murine renal cell carcinoma. *Anticancer Res.* **2001**, *21*, 3845−52. Sawada, S.; Murakami, K.; Yamaura, T.; Mitani, N.; Tsukada, K.; Saiki, I. Therapeutic and analysis model of intrahepatic metastasis reflects clinical behavior of hepatocellular carcinoma. *Jpn. J. Cancer Res.* **2002**, *93*, 190−197.

(16) Noguchi, T.; Yasuda, S.; Itoh, T.; Arai, T.; Kanda, K.; Tsutsui, N.; Nakayama, Y.; Nonogi, H.; Matsuda, T. New multifunctional percutaneous transluminal coronary angioplasty catheter device capable of balloon infusion, local drug delivery and coronary perfusion. *J. Cardiol.* **2000**, *35*, 41−45.

(17) Yasuda, S.; Noguchi, T.; Gohda, M.; Arai, T.; Tsutsui, N.; Nakayama, Y.; Matsuda, T.; Nonogi, H. Local delivery of low-dose docetaxel, a novel microtubule polymerizing agent, reduces neointimal hyperplasia in a balloon-injured rabbit iliac artery model. *Cardiovasc. Res.* **2002**, *53*, 481−486.

(18) Yasuda, S.; Kanna, M.; Sakuragi, S.; Kojima, S.; Nakayama, Y.; Miyazaki, S.; Matsuda, T.; Kangawa, K.; Nonogi, H. Local delivery of single low-dose of C-type natriuretic peptide, an endogenous vascular modulator, inhibits neointimal hyperplasia in a balloon-injured rabbit iliac artery model. *J. Cardiovasc. Pharmacol.* **2002**, *39*, 784−788.

(19) Gottsauner-Wolf, M.; Jang, Y.; Penn, M. S.; Kaplan, A.; Ellis, S. G.; Chisolm G. M.; Topol, E. J.; Lincoff, A. M. Quantitative evaluation of local drug delivery using the infusa sleeve catheter. *Cathet. Cardiovasc. Diagn.* **1997**, *42*, 102−108. Suzuki, T.; Kopia, G.; Hayashi, S.; Baily, L. R.; Llanos, G.; Wilensky, R.; Klugherz, B. D.; Papandreou, G.; Nayaran, P.; Leon, M. B.; Yeung, A. C.; Tio, F.; Tsao, P. S.; Falotico, R.; Carter A. J. Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. *Circulation* **2001**, *104*, 1188−1193.

(20)]Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors. Synthesis of water-soluble, topically effective, intraocular pressure-lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? *J. Med. Chem.* **1999**, *42*, 2641−2650. Pouticello, G. S.; Freedman, M. B.; Habecker, C. N.; Lyle, P. A.; Schwam, H.; Varga, S. L.; Christy, M. E.; Randall, W. C.; Baldwin, J. J. Thienothiopyran-2-sulfonamides: A novel class of water-soluble carbonic anhydrase inhibitors. *J. Med. Chem.* **1987**, *30*, 591−597.

(21) Knight, C. G.; Willenbrock, F.; Murphy, G. A novel coumarin-labeled peptide for sensitive continuous assay of the matrix metaloproteinases. *FEBS Lett.* **1992**, *296*, 263−266.

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