β-Cyclodextrin tetradecasulfate, a novel cyclic oligosaccharide, inhibits thrombus and neointimal formation after coronary vascular injury

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Background Neointimal formation is a major cause of restenosis after interventional vascular procedures. β-Cyclodextrin tetradecasulfate (β-CDT) has been shown to inhibit fibroblast growth factor activity and we hypothesized that β-CDT would reduce intimal formation.

Design Three studies were performed: (1) pharmacokinetics of oral and intravenous β-CDT and determination of optimal dose, (2) determination of efficacy of oral and intravenous β-CDT in reducing neointimal formation after balloon-overstretch injury and (3) determination of the effect of β-CDT on cellular proliferation, factor Xa activity, activated clotting time, activated partial thromboplastin time and thrombus formation.

Methods Pharmacokinetics were determined in eight domestic swine following administration of oral β-CDT and intravenous β-CDT at three doses each. In the efficacy study, balloon-overstretch injury of 37 pigs (69 arteries) was performed and randomized into three groups (n = 23 arteries/group): control, oral administration of 300 mg β-CDT/kg per day or intravenous infusion of 100 mg β-CDT/kg per day. Animals were sacrificed 14 days later. Cellular proliferation and mural thrombus were determined in six arteries/group at 5 days and endothelial coverage was evaluated at 5 and 14 days.

Results Oral and intravenous β-CDT reduced the intimal hyperplasia area normalized to injury index by 24 and 48%, respectively: control, $3.03 \pm 0.75\,mm^2$, oral, $2.31 \pm 0.83\,mm^2$ ($P = 0.004$) and intravenous, $1.67 \pm 0.73\,mm^2$ ($P = 0.0000002$). β-CDT reduced cellular proliferation (control, 55 ± 18%, oral, 35 ± 7%, $P = 0.03$ and intravenous, 30 ± 12%, $P = 0.01$) and mural thrombus formation (control, $0.84 \pm 0.4\,mm^2$, oral, $0.44 \pm 0.14\,mm^2$, $P = 0.04$, intravenous, $0.42 \pm 0.09\,mm^2$, $P = 0.03$). Endothelial coverage was increased in the experimental groups ($P = 0.008$, oral versus control, $P < 0.0001$, intravenous versus control). Factor Xa activity was inhibited 9–10 fold following intravenous administration while oral administration demonstrated no effect.

Conclusions Both oral and intravenous formation of β-CDT reduced intimal hyperplasia with the greatest reduction in the intravenous group. We postulate that β-CDT was effective by the combination of increasing endothelial coverage, reducing mural thrombus formation, inhibiting factor Xa activity and reducing cellular proliferation. *Coron Artery Dis* 13**:**189–197

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Introduction

Neointimal hyperplasia remains the major cause of long-term morbidity of interventional vascular procedures. Following stent implantation exuberant intimal formation results in a restenosis rate of 20–32% [1,2] and reintervention to treat restenosis is associated with increased morbidity and mortality.

Fibroblast growth factor (FGF) plays an important role in mesenchymal cell migration, proliferation and differentiation and extracellular matrix deposition following vascular injury [3–6]. Smooth muscle cells (SMCs) express both basic FGF (bFGF) and the FGF receptor-1 after balloon injury and bFGF is released from extracellular matrix binding sites [4,7]. Administration of bFGF after vascular injury results in increased intimal thickening [4,5,8–10] and animals receiving anti-bFGF immunoglobins after balloon injury have reduced intimal/medial ratios [6] and SMC proliferation rates [3].

β-Cyclodextrin tetradecasulfate (β-CDT) is a soluble monomeric cyclic oligosaccharide consisting of seven glucose units arranged in a rigid toroidal structure containing 14 sulfate groups. Polysulfated cyclodextrins possess various cellular activities that result, in part, in an inhibition of SMC proliferation and promotion of endothelial cell proliferation. Previous studies from our laboratory have demonstrated that biological activity is related to sulfate density, as the presence of 9–10 sulfate groups on the cyclodextrin molecule is associated with 100% SMC inhibition while less than eight sulfates is associated with only 10% inhibition [11]. The sulfate groups confer a critically high negative charge density resulting in a strong affinity and complexation with FGF as demonstrated by biaffinity chromatography [11–13]. Subsequent studies demonstrated that there is a very rapid association rate (0.34 ± 0.07/min) and a very slow dissociation rate ($3.3 \pm 0.2 \times 10^{-7}$/min) suggesting a high-affinity interaction between bFGF and β-CDT and indicating an ability of β-CDT to rapidly sequester FGF [14]. A dose-dependent shift of the FGF equilibrium concentration on cell receptors results, leading to an alteration of FGF function [13], thereby inhibiting SMC proliferation and intimal growth [11,15]. Binding of β-CDT with acidic FGF stimulates endothelial cell proliferation *in vitro* [12,16] that in turn may contribute to a reduction in intimal thickening [17]. In rats extravascular polymeric β-CDT decreases intimal thickening but is inflammatory [14]. We hypothesized that a non-polymeric β-CDT delivered systemically would attenuate neointimal formation following vascular injury in porcine coronary arteries without vascular inflammation and sought to elucidate the mechanism governing this effect.

Methods

Pharmacokinetic studies

All procedures were in accordance with American Heart Association guidelines and the Institutional Animal Care and Use Committee of the University of Pennsylvania approved the protocol. Selection of the optimal oral and intravenous doses for the subsequent efficacy studies was performed in eight domestic swine (25–30 kg in weight). Continuous intravenous access was achieved via the jugular vein. Oral β-CDT (Cerestar USA, Inc., Hammond, Indiana, USA) was administered to non-sedated animals in the following doses: 20 mg/kg, 200 mg/kg or 300 mg/kg. Intravenous β-CDT was administered via jugular venous access in 2.5 mg/kg, 20 mg/kg or 100 mg/kg boluses. Blood samples were obtained prior to the bolus and 0.5, 1, 1.5, 2, 4, 8, 12, 18 and 24 h after administration.

Complexation of β-CDT with cationic dye results in a spectral shift known as metachromasia, which can be exploited to measure plasma levels of the compound [18]. Citrated blood samples were centrifugated at 2000 rpm, 4°C, for 15 min and serum was diluted 1 : 100 and 1 : 400 in distilled water to avoid the interference of proteins of relatively high ionic strength. Metachromasia with 5 μg/ml Azure A dye solution (Fisher Scientific Co., Fairlawn, New Jersey, USA) was analyzed at a 620 nm wavelength [11] (UV/Vis-Spectrophotometer Bio 20, Perkin Elmer, Shelton, Connecticut, USA) to determine (β-CDT) concentration.

Factor Xa activity was measured using a calorimetric assay (Diagnostica Stago, Asnieres-sur-Seine, France) based on in-vitro factor Xa inhibition by antithrombin–heparin complexes [19]. Activated clotting time (ACT) was determined using the Hemochron system (International Technidyne Corp., Edison, New Jersey, USA), which tests the timing of clot formation of whole blood using kaolin as an activator [20]. Activated partial thromboplastin time (APTT) was measured using the Platelin Activator Assay Kit (General Diagnostics, Morris Plains, New Jersey, USA) for evaluation of activation of the intrinsic system [21].

Efficacy study

Swine were randomized into three arms (23 arteries/group): (1) control, (2) oral administration or (3) intravenous infusion. Additional animals (six arteries/group) were sacrificed at 5 days to evaluate cellular proliferation, thrombus and endothelial coverage. β-CDT was started 3 days before coronary injury and continued until sacrifice. Continuous intravenous infusion utilized an indwelling jugular venous catheter connected to a peristaltic pump (Harvard Apparatus Company, Cambridge, Massachusetts, USA). Since β-CDT is an oligosaccharide, serum glucose levels were measured. Mg^{2+} concentration was monitored because β-CDT is a highly negatively charged molecule and complexation with cations may occur. ACT, APTT and factor Xa activity were also measured before instigation of β-CDT, 3 days after initiation and at the end of the study (17 days).

The day prior to the procedure, the animals were administered 325 mg of aspirin followed by 81 mg/day until sacrifice. The pigs were anesthetized with intramuscular ketamine (22 mg/kg), xylazine (5 mg/kg) and atropine (0.2 mg/kg). After intubation, ventilation and anesthetic control was achieved with oxygen and isoflurane (2.5%). Continuous electrocardiographic and arterial blood pressure monitoring were performed. An 8 French sheath was placed in the femoral artery and heparin was administered to achieve an ACT > 300 s. Left coronary angiograms were obtained using an 8 French hockey stick guiding catheter. Vascular injury was caused by three 60 s balloon inflations using a 3.5 mm angioplasty balloon in the left anterior descending and circumflex arteries to achieve a balloon-to-artery ratio of 1.25 : 1. Following the procedure the sheath was removed, the artery ligated, the muscle layers and skin sutured and the animal was brought back to its cage.

Sacrifice was achieved by an overdose of pentobarbital and potassium chloride. The heart was immediately removed and the coronary arteries were perfusion-fixed at 100 mmHg with 10% neutral-buffered formalin. Balloon-injured arterial segments were dissected *en bloc* from the epicardial surface. Serial 2–3 mm arterial sections were embedded in paraffin and cut into 5 μm thick sections. Tissue sections were stained with hematoxylin/eosin and Masson's trichome/Verhoeff–van Gieson elastic stains.

Morphometric analysis

Morphometric analysis was performed only on those arterial sections without side branches, which demonstrated an internal elastic lamina (IEL) fracture. Measurements were performed using a light microscope fitted with a computer-interfaced image analyzer (Bioquant System IV, R&M Biometrics Inc., Nashville, Tennessee, USA and Summa Sketch II Plus, Summagraphics Corp., Seymour, Connecticut, USA). The following parameters were measured: luminal area, vessel area, intimal area (IA), IEL length, fracture length (FL) and maximal intimal thickness. The extent of injury, defined as the injury index, was calculated as the ratio of FL/IEL length. From these measurements two indications of the vessel response to injury were derived: the mean IA of all sections with IEL fraction, normalized to the injury index (IA/injury index) for the vessel, and correlation of IA and injury index.

Arterial sections from the subset of animals euthanized at day 5 were assessed for mural thrombus, defined as intimal or retromedial thrombus or remnants of an original thrombus undergoing organization [22]. If present the area of thrombus was measured and the results expressed as thrombus area normalized to injury index.

Immunohistochemistry

Arterial sections were deparaffinized and fixed with 4% paraformaldehyde in phosphate buffer (0.01 mol/L, pH 7.4), permeabilized using 0.2% Triton and blocked with 3% bovine serum albumin to reduce non-specific binding. Quantification of cellular replication by proliferating cell nuclear antigen (PCNA) utilized fluorescein isothiocyanate (FITC)-conjugated anti-PCNA mouse monoclonal PC10 antibody (1 : 100, Sigma Chemical, St Louis, Missouri, USA). The tissues were counterstained with Hoechst counterstain 33258 (Sigma Chemical) and visualized with fluorescent microscopy. The proliferation index was expressed as the ratio of PCNA positive to total nuclei in the medial layer adjacent to the area of injury. A mean proliferation index was determined from at least four sections within each artery. The arterial proliferation indices for each artery were then averaged for each treatment group (six arteries/group) and the groups compared. A section of tonsil served as an internal positive control. Quantification of endothelial coverage at the site of injury at 5 and 14 days utilized mouse monoclonal antihuman Von Willebrand factor (1 : 50, Dako Corporation, Carpinteria, California, USA). Endothelial coverage is reported as the percentage of the vascular luminal circumference lumen and neointimal area. Mean percentage coverage was calculated for each artery and the group means compared.

Adverse events and toxicity analysis

Unanticipated bleeding events were recorded. Hematoxylin- and eosin-stained sections of the liver, kidney and heart were analyzed in a blinded fashion by an experienced pathologist and evaluated for microscopic changes, with particular attention being paid to the presence of fibrosis or inflammation.

Statistical analysis

The sample size of 23 arteries/group was predetermined to ensure a power of 90%, and a two-sided level of significance of 0.05. to detect a 40% reduction in neointimal area in the treated groups, assuming a control neointimal area of $0.5 \pm 0.25\,mm^2$. All results are expressed as mean ± 1 SD. Categorical data analyses (adverse events) were performed using the χ^2 test and the Fisher exact test (2×2 table). One-way analysis of variance (ANOVA) was used to compare the morphometric data of the β-CDT-treated and -untreated arteries. If the F-test results were significant, Bonferroni's analysis was used for multiple comparisons. To compare differences in the slopes of graphical comparisons, linear regression was performed. A value of $P < 0.05$ was considered significant and all reported P values are two sided.

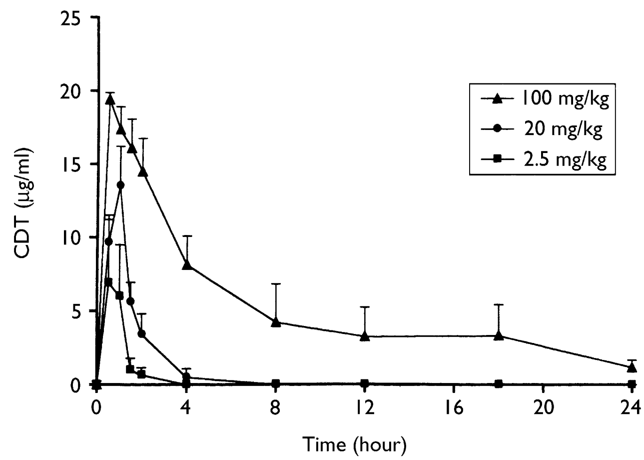
Results

Pharmacokinetics of β-cyclodextrin tetradecasulfate

Following the 20 mg/kg oral dose β-CDT was not detected in plasma. Oral doses of 200 and 300 mg/kg resulted in elevated β-CDT plasma levels for up to 18 h, with peak plasma concentrations of 0.5 and 0.6 μg/ml at 4–6 h. No elevation in APTT or ACT was noted following oral β-CDT. Intravenous administration of the 2.5 or 20 mg/kg bolus resulted in a rapid increase and subsequent decrease in plasma concentration to below the detection limit within 4 h. The 100 mg/kg bolus resulted in a prolonged increase in plasma levels with elimination over 20–24 h (Fig. 1). After intravenous administration the APTT and ACT levels were elevated and significantly correlated with the β-CDT plasma levels ($r^2 = 0.83$ and 0.82 respectively, $P = 0.0001$).

The oral dose chosen for evaluation was 300 mg/kg per day administered as a single dose. The intravenous dosing regimen was selected to ensure a consistent ACT of 180 s over the duration of the study. This corresponded to a serum level of 10 g/ml achieved with a continuous infusion rate of 100 mg/kg per day.

Fig. 1



Pharmacokinetics of a single bolus of intravenous β-cyclodextrin (CDT) tetradecasulfate.

Activated clotting time, factor Xa activity and glucose and magnesium levels

In the efficacy study the ACT was not elevated in the control and oral groups (Table 1). In the intravenous group the mean ACT was consistently elevated; on day 3 it was 189 ± 15 s and on day 17, 176 ± 18s. Factor Xa activity (IU/ml) was unchanged in the control and oral groups. In the intravenous group, there was 9–10-fold inhibition of factor Xa activity on days 3 and 17 (Fig. 2). Serum glucose and magnesium concentration did not significantly differ between groups at any time point.

Morphometry

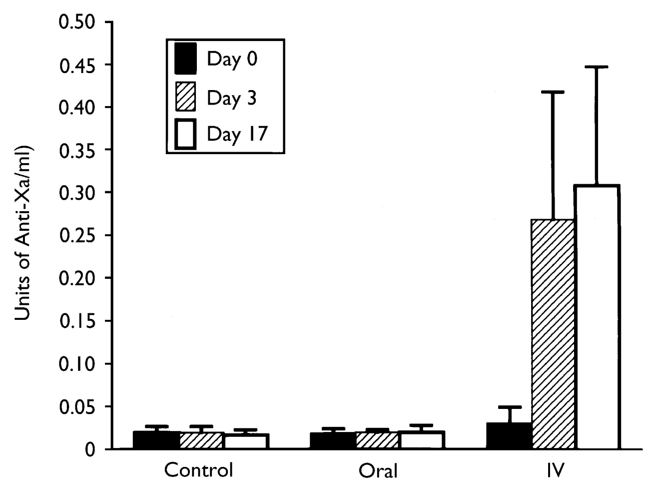
The mean balloon-to-artery ratios were similar in all groups (control, 1.29 ± 0.11, oral, 1.28 ± 0.14 and intravenous, 1.28 ± 0.11, $P =$ NS). In total, 281 injured arterial sections were evaluated. None demonstrated a total occlusion. The luminal area was smallest in the control group (Table 2). Similarly, the vessel areas were greater in the experimental groups but the differences did not reach statistical significance. The injury index was greater in the intravenous group (control, 0.20 ± 0.11, oral, 0.21 ± 0.10 and

Table 1 Activated clotting time and glucose and magnesium levels

	Control	Oral	Intravenous
ACT (s)			
Baseline (day 0)	102 ± 26	120 ± 25	107 ± 21
Peri-procedure (day 3)	103 ± 30	122 ± 30	189 ± 15
At sacrifice (day 17)	107 ± 21	83 ± 17	176 ± 18
Glucose (mg/dl)			
Baseline (day 0)	83 ± 16	78 ± 13	87 ± 13
Peri-procedure (day 3)	83 ± 16	99 ± 12	100 ± 11
At sacrifice (day 17)	107 ± 10	103 ± 28	118 ± 22
Mg^{2+} (mg/dl)			
Baseline (day 0)	2.00 ± 0.23	2.10 ± 0.18	2.13 ± 0.24
Peri-procedure (day 3)	2.00 ± 0.27	2.05 ± 0.17	1.99 ± 0.15
At sacrifice (day 17)	2.26 ± 0.19	2.32 ± 0.22	2.33 ± 0.39

The animals were not fasted prior to obtaining the final samples. ACT, activated clotting time.

Fig. 2



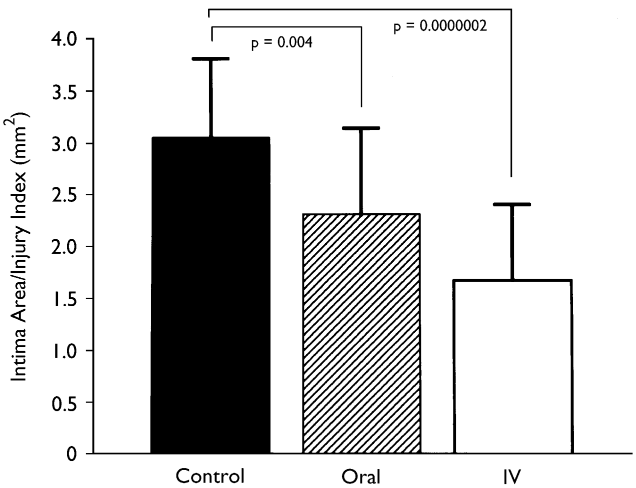
Temporal effects of β-CDT on factor Xa activity. Day 0 reporesents the fator Xa level prior to the first administration β-CDT

Table 2 Morphometric analysis of injured vascular segments

	Control ($n = 23$)	Oral ($n = 23$)	Intravenous ($n = 23$)
Lumen area (mm^2)	1.82 ± 0.43	1.98 ± 1.48	2.02 ± 0.64
Vessel area (mm^2)	3.07 ± 0.37	3.22 ± 1.53	3.26 ± 0.84
Maximal intimal thickness (mm)	0.42 ± 0.11	0.34 ± 0.15*	0.29 ± 0.10**
Intimal area (mm^2)	0.62 ± 0.23	0.50 ± 0.27	0.39 ± 0.21***
Intimal area/injury index (mm^2)	3.03 ± 0.75	2.31 ± 0.83†	1.67 ± 0.73††

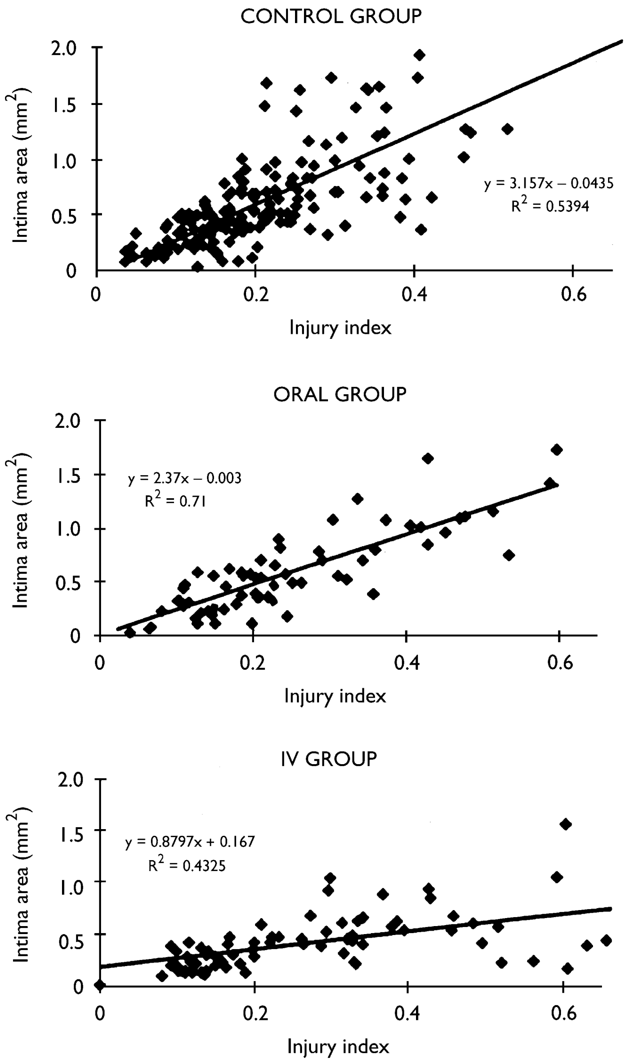
*$P = 0.06$, versus control; **$P < 0.001$; ***$P = 0.003$; †$P = 0.004$, versus control; ††$P = 0.0000002$, versus control.

Fig. 3



Effect of oral and intravenous β-CDT on normalized intimal area.

Fig. 4



The relationship between injury index and intimal area. The significant decrease in slope between treated and control groups indicates that both oral ($P = 0.001$) and intravenous ($P < 0.0000002$) β-cyclodextrin tetradecasulfate administration resulted in less intimal growth despite similar degrees of arterial injury. Each data point represents a single injured arterial segment.

intravenous, 0.26 ± 0.09, $P = 0.07$). Maximal intimal thickness and the IA were significantly decreased in the β-CDT groups. Oral β-CDT resulted in a 24% decrease in IA, compared with control ($P = 0.004$, Table 2), while intravenous β-CDT resulted in a 45% decrease ($P = 0.0000002$, versus control, Fig. 3). The correlation between neointimal response and injury was statistically significant for each study group (Fig. 4). However, the slopes significantly differed between the control and oral ($P = 0.001$) and the control and intravenous groups ($P = 0.0000002$), confirming the effect of β-CDT in reducing IA. Representative photomicrographs are shown in Figure 5.

Effects on thrombus formation, endothelial coverage and cellular proliferation

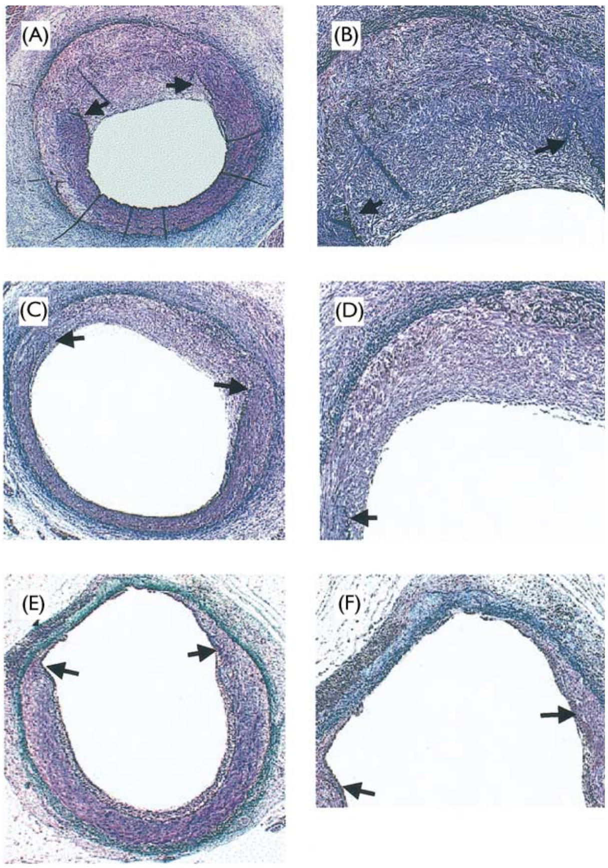
Thrombus area was reduced in the β-CDT-treated arteries (Fig. 6). Oral β-CDT resulted in a 47% reduction of thrombus area compared with control (0.44 ± 0.14 versus $0.84 \pm 0.4\,mm^2$, $P = 0.04$) while a 50% decrease was observed after intravenous administration ($0.42 \pm 0.09\,mm^2$, $P = 0.03$). No artery exhibited endothelial coverage of the neointima at 5 days. At 14 days the percentage of the arterial lumen covered by endothelial cells was increased in the experimental groups compared with control (control, $80.0 \pm 15.3\%$, oral, $90.8 \pm 9.1\%$ and intravenous, $95.6 \pm 5.5\%$; $P = 0.008$, oral versus control, $P < 0.0001$, intravenous versus control). The neointima also had greater endothelial coverage following β-CDT administration (control, $55.6 \pm 31.4\%$, oral, $83.6 \pm 15.9\%$ and intravenous $92.8 \pm 8.5\%$; $P = 0.001$, oral versus control, $P < 0.0001$, intravenous versus control). The percentage of PCNA-positive medical cells (that is, proliferation index, Table 3), was significantly higher in the control ($55 \pm 18\%$) than in the oral ($35 \pm 7\%$, $P = 0.03$, 36% reduction) and the intravenous ($30 \pm 12\%$, $P = 0.01$, 45% reduction) groups.

Adverse events and toxicity of β-cyclodextrin tetradecasulfate

No animal in the pharmacokinetic study suffered any complication, including bleeding, and the post-procedural course was uneventful in all control and orally treated pigs. In the intravenous group bleeding was appreciated at surgical vascular access sites (femoral artery or the indwelling jugular venous catheter) in six of 19 pigs (32%, $P < 0.03$). One of these animals died from femoral arterial bleeding and one required a blood transfusion. Most bleeding (four of seven pigs) occurred within the initial 24 h following angioplasty in the setting of extremely elevated ACT values (>450 s) resulting from the administration of 4000 IU of heparin during the procedure. The heparin dose was then adjusted to achieve an ACT of $\pm$300 s and only two minor, transient bleeding episodes were observed thereafter (of 12 pigs, 17%). Bleeding ceased after the infusion was discontinued for 3 h and did not reoccur in any animal. All data from all animals, except the premature death, were used for further analysis.

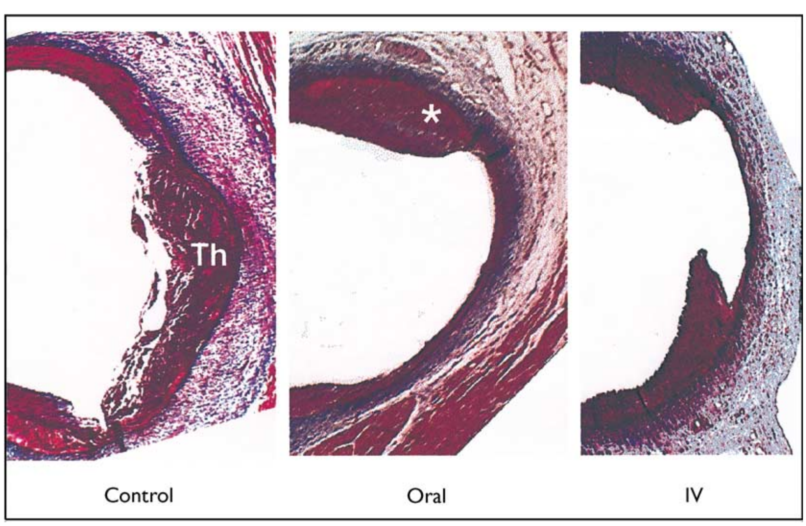
No fibrosis or inflammation was observed in liver and kidney tissue samples of animals treated with β-CDT. The arterial wall perivascular area and the myocardium likewise revealed no pathologic changes.

Fig. 5



Representative cross-sections of arteries obtained 14 days after injury showing decreased intimal area in the oral and intravenous groups. In the intravenous sections the only intima noted is retromedial. Arrows point to the ends of the fractured internal elastic lamina. (A) and (B) are from the control group, (C) and (D) are examples of the effects of low-dose, orally administered β-cyclodextrin tetradecasulfate (β-CDT) and (E) and (F) are examples of continuous intravenous administration of β-CDT. Photomicrographs on the left are at 40x magnification and those on the right are at 100x magnification.

Fig. 6



Representative arterial sections obtained 5 days after arterial injury. Reduced thrombus formation is noted in arteries obtained from animals treated with β-CDT. No thrombus is noted in the section from the intravenous group. Each arterial section is at 100x magnification. Th, thrombus; *, retromedial thrombus in sections obtained after oral administration.

Discussion

β-CDT reduced neointimal hyperplasia in a dose-dependent fashion. A greater effect was observed following intravenous administration (serum level, 10 g/ml) than following oral administration (peak serum level, 0.6 µg/ml,

Table 3 Effects of oral and intravenous β-cyclodextrin tetradecasulfate compared with control levels

	Oral	Intravenous
Serum level	0.6 μg/ml	10 μg/ml
Reduction in normalized intimal area (14 days)	24%	48%
Factor Xa inhibition (14 days)	0%	9–10x
Increase in endothelial coverage of neointima (14 days)	149%	158%
Reduction in thrombus area (5 days)	47%	50%
Reduction in cellular proliferation (5 days)	36%	45%

Table 2). The efficacy of β-CDT is likely to have resulted from a combination of accelerated endothelial coverage, reduction in mural thrombus formation and reduced cellular proliferation. The greater benefit observed with intravenous β-CDT compared with oral formulation most likely reflects a more substantial reduction of cellular proliferation. As such the current study demonstrates significant efficacy of β-CDT in inhibiting intimal hyperplasia in a large animal model and demonstrates for the first time an effect of β-CDT on coagulation, including factor Xa activity, resulting in a reduction in thrombus formation after vascular injury.

Thrombus, located with dissection planes and the arterial lumen, is present following angioplasty injury and the resulting thrombus can act as a bioabsorbable matrix into which cells migrate, proliferate and synthesize extracellular proteins [23]. Indeed, thrombus may play a role in subsequent intimal development [24] and, as demonstrated in the current study, reduced mural thrombus is associated with reduced intimal formation. In addition, coagulation proteins trapped within the thrombus such as factor X, factor Xa, thrombin and fibrin fragments have been implicated as SMC mitogens [24,25]. Inhibition of factor Xa with recombinant tick anticoagulant peptide (rTAP) for 60 h reduced intimal formation for up to 28 days in the porcine coronary stent model [26] while a 2 h infusion of antistatin or rTAP reduced restenosis in atherosclerotic rabbit femoral arteries [27]. Like rTAP, β-CDT inhibits factor Xa activity. The greater inhibition of factor Xa activity achieved with the intravenous than the oral formulation may be responsible for the more profound antimitogenic effect, as reflected by the additional 25% inhibition in cellular proliferation noted in the intravenous group. The antiproliferative effect observed with β-CDT may have also been mediated, in part, through indirect effects on thrombin activity. Thrombin is a mesenchymal mitogen and is chemotactic for monocytes; properties independent of its anticoagulative effects [28]. Others have demonstrated that the selective thrombin inhibitor hirudin reduces restenosis in atherosclerotic rabbit femoral and porcine coronary arteries [22,29]. Administration of an antibody to the thrombin receptor reduced neointima in the absence of an effect on platelet aggregation, prothrombin time or APTT [30].

The multiplicity of effects of β-CDT on neointimal formation is not unexpected since polyanionics such as glycosaminoglycans do not have specific biological activities themselves but effect cellular processes by modification via complexation. Cationic proteins, such as FGF, have several biological actions and the anticipated binding of β-CDT to FGF would be expected to yield multiple effects. bFGF acts synergistically with, or is induced by, thrombin to stimulate vascular SMC proliferation [31]. Hence, β-CDT binding to bFGF may have effectively prevented thrombin-mediated cellular proliferation. That the intravenous formulation of β-CDT more effectively inhibited neointimal formation may be due to increased inhibition of factor Xa activity and potentially increased bFGF binding at the higher serum levels afforded by intravenous administration, which, in turn, may account for the more potent effect on cellular proliferation. In addition, more rapid re-endothelialization with the IV preparation may have suppressed neointimal hyperplasia. The effect of β-CDT on the rate of SMC apoptosis is unknown.

The similar inhibition of mural thrombus by both oral and intravenous β-CDT, despite differing effects on ACT, APTT and factor Xa levels, is surprising although not entirely unexpected. Previous studies have shown preferential vascular binding of heparin with more than $1000\times$ greater concentration on the endothelial surface than in the plasma [32]. Although systemic bioavailability of orally administered heparin may approximate 0.5%, this low serum level has been associated with a substantial reduction of thrombus formation in a rat jugular vein preparation [33,34]. A similar finding has also been shown in humans for dextran sulfates [35]. β-CDT, while being similarly ionic in character, possesses an almost 10-fold smaller molecular size, which increases avidity for the endothelium. As such, selective endothelial binding after oral dosing may have resulted in decreased thrombus formation in the absence of an elevation in systemic coagulation markers.

The observation that β-CDT reduced thrombus formation and at higher doses demonstrated anticoagulative properties indicates that β-CDT may be a potential treatment for thromboembolic as well as vascular diseases resulting from exuberant intimal hyperplasia. β-CDT demonstrated predictable pharmacokinetics (in this model) and is relatively inexpensive to manufacture. Continuous intravenous administration was associated with increased bleeding but this reflects the relatively high doses of heparin administered during the procedure. Nonetheless, the increased incidence of bleeding in the intravenous group may make it necessary to evaluate lower doses. Importantly, the demonstration that oral β-CDT resulted in no increase in the APTT, ACT or anti-factor Xa activity while reducing cellular proliferation and thrombus formation indicates that oral administration is a safe therapeutic approach to intimal hyperplastic diseases.

There are several limitations to this study. Although previous studies have demonstrated strong binding of β-CDT to FGF we did not evaluate the intravascular effects of β-CDT on FGF levels. Furthermore, the effect of oral dosing may have been underestimated since plasma concentration of β-CDT after oral administration peaks at 4–6 h thereby the potential anticoagulative and antiproliferative effects of oral β-CDT may have suffered due to once-a-day dosing rather than a multiple dosing regimen. However, given the preferential binding of polyanionic substances such as β-CDT, persistent binding to the endothelial surface may have been present. Also, this study demonstrated the efficacy of β-CDT in reducing intimal hyperplasia at 14 days after injury and it is unknown whether a more prolonged effect would be observed. It is anticipated that the reduction in intima would be present at later time points since cellular proliferation declines after 7 days and little increase in IA has been noted between 14 and 28 days [36]. In addition, previous studies have shown that 14 days of continuous thrombin inhibition with hirudin is associated with a decreased IA 28 days after injury [22].

In conclusion, this study demonstrates that β-CDT reduced intimal hyperplasia after balloon-overstretch injury in porcine coronary arteries. The action of β-CDT as demonstrated in this model was a reduction of mural thrombus formation, an increase in endothelial coverage of the arterial wall and a reduction in cellular proliferation, possibly secondary to decreased factor Xa activity.

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