ODN (18mer) was injected with the PB (n = 7) at 4 atm inflation pressure for 30 s: with the IO (n = 3) ODN was applied with 7.5 mA current for 3 × 60 s. Parallel studies were performed using rhodamine labelled ODN (rhoODN) for histological localization. Samples were harvested at 30 min, 2 h, 24 h and 7 d and processed for scintillation counting or fluorescence microscopy.

Results:

		μg ODN per artery			
		30 min	2 hours	24 hours	7 days
PB	570 μg/ml	3.45 ± 1.3	1.42 ± 0.65	0.08 ± 0.02	ND
IO	855 μg/ml	7.3 ± 2.4	1.5 ± 0.6	0.52 ± 0.35	0.26 ± 0.11

n = 3/group; data are mean ± SEM

While IO appeared to deliver more than PB acutely, there was rapid washout at 2 h in both groups. However, after 24 h persistence appeared better with the IO, and in one sample there was persistence at 7 d with IO. This may have been in part due to improved tissue distribution and cellular uptake with IO, which was detected with rhoODN.

Conclusion: Iontophoresis appears superior to PB for LD of ODN in pig coronary arteries and may be feasible for restenosis prevention in the clinical setting.

938-38 Acute Arterial Occlusion and Platelet Deposition Are Markedly Reduced by Local Delivery of a Novel Nitric Oxide Donor

Myung Ho Jeong, Whyte G. Owen, Amir Lerman, David N. Fass, Jean Gregoire, Michael E. Staab, Sanjay S. Srivatsa, David R. Holmes, Robert S. Schwartz. *Mayo Clinic, Rochester, MN 55905*

Nitric oxide (NO) is an endothelial-derived vasodilator and anti-platelet aggregation factor. We studied local delivery of a novel NO donor in pigs after severe carotid artery crush injury. *In vivo* quantitative platelet deposition (PD) was measured by 111-In tropolone labeled platelets, and normalized blood flow (NBF) measured by Doppler flowmetry. Local arterial infusion of the NO donor dansylpiperazine nonoate (GLO/NO) was compared with control (saline), infused identically by the Dispatch catheter. Four groups were studied: *A* (10 arteries): injury, local saline, *B* (10 arteries): injury, local GLO/NO (10 ml/3 × 10^{-4} mol over 10 min), *C* (9 arteries): injury, systemic GLO/NO (3 × 10^{-4} mol), *D* (6 arteries): injury, systemic GLO/NO, local saline. Each was studied at 1 and 24 hours.

Group	A		B		C		D	
Time (hr)	1	24	1	24	1	24	1	24
Patency (%)	80	60	90*	90#	33*	11#	83	66
NBF	0.25*	0.26*	0.73*	0.75*	0.18*	0.07*	0.59	0.13
PD (× 10^5/cm^2)	5.5 ± 2.2*		1.3 ± 0.4*		4.9 ± 1.0*		7.6 ± 3.1	

*p < 0.05: Group A vs B, Group B vs C, #p < 0.005: Group B vs C

Conclusion: Local NO delivery significantly reduces occlusion and platelet deposition after severe arterial injury. This underscores the role of NO as a modulator of vascular tone and thrombus formation, and suggests an effective therapeutic strategy following angioplasty.

938-39 In Vitro Model to Investigate Stent Activated Platelets by Flow Cytometry

Christian Beythien [1], Kai Gutensohn [2], Wolfram Terres [1], Christian W. Hamm [1], Peter Kühnl [2], Thomas Meinertz [1]. [1] *Dept. of Cardiology, University Hospital Eppendorf, Hamburg, Germany;* [2] *Dept. of Transfusion Medicine and Transplantation Immunology, University Hospital Eppendorf, Hamburg, Germany*

The application of stents in coronary arteries is still limited by acute or subacute occlusion. Activated platelets play a major role in thrombus formation. In an in vitro model of stent thrombosis we investigated the expression of activation dependent glycoproteins (GP) on platelets by flow cytometry. Tantalum wire stents (n = 12) were placed in one of two parallel silicon tubings with circulating citrated platelet rich plasma of healthy and drug free volunteers. Over 10 minutes aliquots of platelet rich plasma were drawn via a three way faucet in two minute intervals. Blood samples were immediately fixed with glyoxal, paraformaldehyde and buffer. For flow cytometric analysis monoclonal antibodies CD41a (GPIIb/IIIa), CD42b (GPIb), CD62 (GMP-140) and CD63 (GP53) were used. 10,000 events were acquired in a life gate setting. Results were expressed as "platelet activation" (PA; Rinder, Transfusion 33, 1993). Within 2 minutes after the start of circulation, the expression of CD62 and CD63 increased in the tubing system with the stent. Over 10 minutes platelet activation progressively increased; CD62 1051 ± 548 vs 359 ± 358 PA control without stents ($p < 0.01$) and CD63 981 ± 355 vs 428 ± 156 PA ($p < 0.005$). Antigens CD41a and CD42b did not show significant changes. Thus, in our in vitro model there is activation of platelets by stents probably caused by shear stress and contact to the artificial surface. Flow cytometry is a diagnostic tool to quantify platelet activation, and may help to improve stent material and design.

938-40 Photodynamic Therapy and Local Drug Delivery in a Restenosis Model

Peter Gonschior, Clemens Pahl, Christoph Abels [1], Berthold Höfling. *Med. Department I, Klinikum Großhadern;* [1] *Inst. Surg. Research, University of Munich, Germany*

Photodynamic therapy (PDT) was examined in porcine restenosis model. Unidirectional injury of media by a atherectomic catheter (UI) was used. The photosensitizer, Photofrin (PF; QLT, Canada) was delivered with a new needle injection catheter (NIC) and activated by adequate monochromatic light irradiation. 46 arterial segments from pigs (mean weight 34 kg) underwent UI and were removed at 7, 14 or 21 days. 16 vessel segments received no further treatment (Group 1); 12 segments received UI and local drug delivery or monochromatic light exposure. (Group 2 and 3) 28 segments underwent UI followed by selective application of 5 mg PF per treated segment and exposure to monochromatic light with a light delivery catheter (630 nm) with 100 J/cm2 (Group 4, therapeutic group). All vessels were explanted and processed for immunohistochemistry and electron microscopy.

Group 1: An intense inflammation with polymorphonuclear leukocytes and subsequent proliferation of myofibroblasts (maximum after 7 d) was found. After substantial vessel injury only (Group 1), a myoproliferative response resulted in tissue hyperplasia of 1.8 ± mm^2. Group 2 and 3 results did not reveal significant differences to group 1. In media-injured vessels treated with PDT, no inflammation and or proliferative response resulted (area of tissue hyperplasia 0.3 mm^2). A marked destruction of nuclear membranes with PF deposits in smooth muscle cells with cytoplasmatic vacuoles were seen after PDT. These results were only seen with laser application after PF delivery. There were no alterations in nuclear morphology.

Thus, after selective application of a photosensitizer, local PDT led to a marked reduction of proliferation and tissue hyperplasia in a porcine model of restenosis without adverse effects.

938-41 Efficiency of Coronary Drug Delivery With the Needle Injection Catheter

Peter Gonschior, Clemens Pahl, Katharina Larisch, Berthold Höfling. *Medical Department I, Klinikum Großhadern, University of Munich, Germany*

Local delivery of antiphlogistic/proliferative agents might prevent or protract restenosis development. To date local drug delivery is hampered by low local efficiency and adjacent tissue contamination. A new designed over-the-wire needle injection catheter (NIC) for coronary application was used. C^{14}-labelled Carvedilol (molecular weight 400) or Benzoporphyrinderivative (BPD) was tested in normal coronary and femoral porcine arteries (n = 19). The NIC system was used for drug delivery of either 0.3 mg or 0.03 mg C^{14}-Carvedilol (Group 1) or 2 mg BPD (Group 2). Vessels were removed up to 4 hours after drug delivery. Determination of drug content was performed after homogenisation of vessel tissue in ascintillation counter (combustion) or by chromatography. There were no significant differences in the results between 0.3 mg or 0.03 mg C^{14}-Carvedilol or in the analyzation methods (Group 1 or 2). The maximum content could be detected at 2 hours (14.2 ± 1.2% of total drug amount) in perivascular tissue (media 1–2.5%) with a decrease thereafter (to 2.9 ± 01%). There was contamination of perivascular tissue, as ascertained by enhanced drug content (maximum 0.39% of total drug amount immediately after drug delivery). No systemic content was measured after local drug delivery with the NIC; there was no measurable renal, hepatic or splenic drug content.

In conclusion, local drug delivery into arterial vessel wall is feasible using the new needle injection catheter system. It allows a higher efficiency, with prolonged delivery compared with data available relating to other local drug delivery systems. Adjacent tissue contamination must be taken into consideration.

938-42 Local Delivery of Lipid-Complexed Oligonucleotide to Balloon-Injured Pig Coronary Arteries: Radiolabelling Pharmacokinetic and Correlative Fluorescence Microscopic Analysis

Keith A. Robinson [1], Nicolas A.F. Chronos [1], Elisabeth Schieffer [1], Spencer J. Palmer [1], Gustavo D. Cipolla [1], Peter G. Milner [2], Spencer B. King III [1]. [1] *Andreas Gruentzig Cardiovascular Center of Emory University, Atlanta, GA;* [2] *CV Therapeutics Inc., Palo Alto, CA*

Localized delivery (LD) of conventional drugs, gene constructs, and oligonucleotides (ODN) is a potential means to prevent iatrogenic restenosis and thrombosis after vascular interventions. Problems with this technology in-

clude initial tissue loading, adequate retention with sustained release kinetics, and cellular uptake. To evaluate one approach to LD of ODN, we injected 2 ml of a lipid-ODN solution ([18-mer ODN] = 0.57 mg/ml) into the sites of balloon overstretch angioplasty in coronary arteries of normal pigs, using a porous balloon catheter (USCI Inc.) at 4 ATMs for 20 sec. ODN were labelled with ^{3}H (3.75 μCi/ml) or rhodamine. Samples were harvested at 30 min, and 2 and 24 hr and processed for scintillation counting or fluorescence microscopy.

Time	Concentration of ODN, μg/g tissue		
	uninjured coronary	injured, no ODN	injured + ODN
30 min	0.15	1.79	30.46
2 hr	0.07	0.03	10.06
24 hr	0.03	0.00	0.62

By scintillation counting the percentage delivery of the injectate to the injured coronary artery was 0.31% at 30 min, which decreased to 0.13% at 2 hr and 0.01% by 24 hr. Radioactivity of various organs was at background level except heart, lung, liver, and kidney. Fluorescence microscopy demonstrated localization of ODN at 30 min and 2 hr primarily in the adventitia near the medial rupture; label was scarcely detectable at 24 hr. Only rare instances of intracellular localization were observed.

Conclusion: LD of ODN by porous balloon results in low acute transfer and rapid tissue loss, suggesting poor potential for biological effects. Alternative strategies such as iontophoresis and electroporation are under investigation.

938-43 Reduced Thrombogenicity of Polished and Unpolished Nitinol vs. Stainless Steel Slotted-Tube Stents in a Pig Coronary Artery Model

Sushil Sheth, Frank Litvack, Michael C. Fishbein, James S. Forrester, Neal L. Eigler. *Cedars-Sinai Medical Center, Los Angeles, CA*

The thrombogenicity of slotted-tube stents made of nitinol (polished and unpolished) or stainless steel (Palmaz-Schatz) was assessed in a pig coronary artery model. Six nitinol stents were polished mechanically. Six polished nitinol, six unpolished nitinol and five stainless steel stents (15 mm long) were deployed in the mid portion of LAD of 17 pigs with a 3.0 mm balloon, inflated twice at 6 atm for 20 seconds. Oral aspirin and diltiazem as well as intravenous heparin were administered prior to implantation of the stent. All pigs were euthanized after 6 days. Immediately after sacrifice, the heart was removed and the stented segment of LAD was excised and cut open. The amount of thrombus on each stent was assessed by a semiquantitative grading scale (I: no lumen encroachment; II: lumen encroachment; III: subocclusive thrombus and IV: occlusive thrombus) and by the weight of the dry thrombus. Histologic examination of the stented arterial segments was performed after H&E staining to evaluate vascular injury. *Results:*

Parameter	Stainless Steel	Unpolished Nitinol	Polished Nitinol	ANOVA p-value
n	5	6	6	
Thrombus Weight (mg)	9.2 ± 0.3	1.8 ± 1.3	0.6 ± 0.07	<0.000001
Thrombus Grade (I to IV)	II = 5	I = 5, II = 1	I = 6	<0.0001

Histologic examination of the stented arterial segments showed that all 5 animals in the group with stainless steel stents had a deeper stent strut penetration with diffuse, transmural medial necrosis and thinning, compared to none in the groups with nitinol stents (5/5 vs. 0/12; p = 0.0002).

Conclusions: Both polished and unpolished nitinol stents developed significantly less thrombus in comparison to stainless steel stents of similar design as measured by thrombus weight and thrombus grade in the pig coronary artery model. Polished nitinol stents were found to be even less thrombogenic than unpolished nitinol stents. More severe vascular injury may contribute to the higher thrombogenicity of stainless steel stents in this model.

938-44 Fibrin Coated Stents as a Depot to Deliver RGD Peptide Inhibit Vascular Reaction in Atherosclerotic Rabbit Model

John E. Baker, Victor Nikolaychik, Ahmed Zulich, Richard Komorowski, Nicholas Kipshidze. *Medical College of Wisconsin, Milwaukee, WI*

Despite therapeutic success of intracoronary stent implantation, stenting of small vessels remains a problem. Our previous studies of coating metallic stents with biocompatible compounds such as fibrin demonstrated reduced thrombogenicity and the potential for drug delivery. RGD peptide inhibits the interaction of fibrinogen with platelet membranes. We determined the impact of fibrin coated stents loaded with RGD peptide upon restenosis in an atherosclerotic rabbit model. Coated and uncoated novel radially expandable "PEAK" metallic stents were implanted in contralateral areas of 10 rabbit iliac arteries previously denuded of endothelium and placed on a diet supplemented with 2% cholesterol for 3 weeks. QCA and morphometric analysis data obtained 4 weeks following stent implantation revealed that 1) late loss was significantly less in coated group compared with uncoated group (0.12 ± 0.01 vs 0.45 ± 0.05, $p < 0.01$), and 2) the extent of myointimal hyperplasia as defined by histologic analysis was significantly lower in coated than that of the uncoated stent with minimal smooth muscle proliferation using PCNA or inflammatory cell infiltration. Planimetric analysis of vessel cross sectional area also revealed less hyperplasia in coated versus uncoated stents (3.5 ± 0.9 vs 16.4 ± 2.8, $p < 0.01$). We conclude that RGD loaded fibrin coating inhibits vascular reaction following stent implantation.

939 Restenosis: Basic Insight

Tuesday, March 26, 1996, 9:00 a.m.–11:00 a.m.
Orange County Convention Center, Hall E
Presentation Hour: 10:00 a.m.–11:00 a.m.

939-45 Remodeling and Plaque Formation in Restenosis: Effects of Colchicine and Enoxaparin in the Atherosclerotic Rabbit

Jesse W. Currier, Raja Paladugu, William D. Coats, Jr., David P. Faxon. *University of Southern California, Los Angeles, California*

Colchicine (Colc) and enoxaparin (Enox) reduce restenosis after angioplasty(A) in the hypercholesterolemic rabbit model. This study examines the contributions of arterial remodeling (differences in external elastic lamina (EEL) area) and plaque formation (differences in intima + media (I + M) area), to this reduction. Rabbits underwent iliac artery de-endothelialization and were fed a 1.5% cholesterol diet for 6 weeks, at which time A was performed in arteries with ≥ 50% stenosis. Treatment groups included Colc (0.2 mg/kg/d, n = 12), Enox (10 mg/kg/d, n = 8) and A alone (n = 11). There were no differences in pre-A, post A, or reference minimal lumen diameter (MLD). All groups underwent angiography 4 weeks post A and quantitative histology of the most stenotic section was performed.

	A Alone	Enoxaparin	Colchicine
Angio MLD (mm)	0.6 ± 0.1	1.2 ± 0.1†	1.1 ± 0.6*
EEL area (mm^2)	3.94 ± 0.79	2.88 ± 1.33*	5.24 ± 1.89*
I + M area (mm^2)	3.37 ± 0.68	2.25 ± 1.06†	4.41 ± 1.88

Mean ± SD, *$p < 0.04$, †$p < 0.01$ vs. A Alone

Compared with A alone, Colc arteries had a greater I + M area, but because of a greatly increased EEL area, the lumen was still significantly larger. In contrast, Enox treated arteries showed less remodeling, as the EEL area was smaller. However, because of a much smaller I + M area, the lumen remained significantly larger. Thus, Colc reduces restenosis through enhancing remodeling while Enox reduces restenosis via a reduction in plaque formation. This study demonstrates a differential effect of drugs on two principle processes of restenosis: plaque formation and remodeling.

939-46 The Immediate Early Gene Products of Human Cytomegalovirus Increase Vascular Smooth Muscle Cell Migration

Yi Fu Zhou, Zu Yi Yu, Toren Finkel, Stephen E. Epstein. *NIH, Bethesda, MD*

Increasing evidence suggests human cytomegalovirus (HCMV) infection contributes to the development of atherosclerosis and of restenosis; if it does, then the cellular effects of its immediate early (IE) gene products likely play a central role. Since smooth muscle cell (SMC) migration from media to intima is a crucial event of atherogenesis and restenosis, we determined whether HCMV infection increases this process. HCMV rapidly replicates and produces cytopathic effect (CPE) soon after infecting human SMCs; however, we found that only IE gene products are expressed when HCMV infects rat SMCs (RSMCs) and CPE does not occur. We therefore infected RSMCs with high HCMV titers such that 30–40% of cells expressed IE72 and IE84. Since peak expression occurred 3 days post infection (PI), we compared migration rate of infected and noninfected RSMCs 3 days PI using a modified micro-Boyden chamber: cells are placed in 0.5% FBS in the upper chamber, and in PDGF or 10% FBS in the lower. As previously shown, PDGF (0.1–20 ng/ml) increased migration of SMCs in a concentration-related manner. HCMV significantly augmented the response to PDGF (116 ± 51 cells per high power field migrated vs 64 ± 37; $p < 0.01$). However, HCMV did not significantly alter the response to 10% FBS. *Conclusion:* HCMV infection of SMCs, and specifically HCMV's IE gene products, increase the SMC migration response to PDGF. This finding adds an additional mechanism by which CMV may contribute to the development of neointimal lesions, and thereby provides further evidence suggesting that CMV plays a crucial role in the development of restenosis and of atherosclerosis.