1031-199 **Rapamycin Inhibits Expression of Minichromosome Maintenance Proteins in Vascular Smooth Muscle Cells**

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Background: Rapamycin inhibits proliferation of vascular smooth muscle cells (VSMC) and local delivery of rapamycin from polymer-coated stents represents a novel therapeutic strategy for preventing postangioplasty restonsis. The molecular mechanims by which rapamycin exerts its antiproliferative activity involves an inhibition of G1 →S phase transition. However, the effects of rapamycin on S phase and DNA replication remain to be investigated. In the present study the effect of rapamycin on minichromosome maintenance (MCM) proteins 6 and 7, two proteins essential for initiation of DNA replication, was investigated in rat aortic VSMC.
Methods and results: Rapamycin substantially inhibited mitogen-induced MCM6 and MCM7 mRNA (68.2 ± 5.6 %, 71.2 ± 6.9 % inhibition vs. 10 % FBS after 12 hours treatment with 100 nM rapamycin, n=3, p<0.05) and protein (61.1 ± 8.3 %, 52.7 ± 6.0 % inhibition vs. 10 % FBS after 24 hours treatment with 100 nM rapamycin, n=3, p<0.05) expression in a dose-dependent fashion. Transient transfection experiments revealed that rapamycin inhibited MCM6 and MCM7 promotor activity (58.3 ± 6.8 %, 67.1 ± 8.4 % inhibition vs. 10% FBS after 48 hours treatment with 100 nM rapamycin, n=3, p<0.05), implicating a transcriptional mechanism. MCM6 and MCM7 transcriptional activation is regulated by E2F and activity of a luciferase reporter plasmid driven by four E2F elements was also strongly inhibited by rapamycin (63.1 ± 7.3 % inhibition after 48 hours, n=3, p<0.05). The inhibitory effect of rapamycin on MCM6 and MCM7 was reversed by adenoviral mediated overexpression of E2F, indicating that their downregulation by rapamycin involves an E2F-dependent mechanism.
Conclusion: These observation suggest that rapamycin inhibits MCM6 and MCM7 expression by blocking E2F function which may contribute importantly to the inhibition of VSMC DNA synthesis by rapamycin.

1031-200 **Site Specific Systemic Delivery of Rapamycin With Perfluorobutane Gas Microbubble Carrier Reduced Neointimal Formation in the Porcine Coronary Restenosis Model**

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Hypothesis: Stent based delivery of Rapamycin was shown to inhibit intimal hyperplasia in animal models and to eliminate restenosis in clinical trials. However the potential for long-term adverse effects secondary to the polymeric coatings has been subject of concern. It was previously demonstrated that perfluorobutane gas particles adhere to injured arteries. Additionally perfluorobutane gas enhances cell entry for drugs on the surface of bubbles that come into contact with denuded vessel surfaces. The purpose of this study was to investigate the effects of PGMC-based delivery of Rapamycin on expression of p21 and p27 in vascular tissue and restenosis in porcine arteries after stent implantation.
Methods: Seven pigs underwent stent implantation (3 stents/animal). Four pigs received IV injection of PGMC and 2mg of Rapamycin. Two served as control. Four hours later, 3 pigs were sacrificed and stented segments were analyzed by HPLC and western blot. In chronic experiments, 4 pigs (12 stent sites) were sacrificed at 28 days.
Results: HPLC analysis of plasma samples of treated animals showed minimal presence of Rapamycin, whereas HPLC of the treated arteries demonstrated high concentration of the drug (190-280 mcg). Western blot analysis of the stented vessels demonstrated over-expression of p21 and p27. Morphometry showed that the neointimal was significantly reduced 40% in the Rapamycin/bubble group compared with control (2.84 ±0.84 vs. 4.77 ±1.71 mm^2 respectively, p<0.05). **Conclusion:** In this animal model site-specific systemic delivery of Rapamycin using PGMC resulted in over-expression of p-21 and p-27 in the stented segments and significantly inhibited neointimal formation.

1031-201 **Local Delivery of Irinotecan With Drug-Eluting Stents Inhibits Neointimal Proliferation in Hypercholesterolemic Rabbits**

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Background: Recent studies have shown that local delivery of cytostatic or antineoplastic drugs inhibits neointimal growth after stent implantation. Irinotecan inhibits DNA replication.
Objective: To assess the effect of irinotecan-eluting stents on neointimal growth in the aorta of hypercholesterolemic rabbits.
Methods: Phosphorylcholine (PC) coated stents were deployed in the aorta of 30 hypercholesterolemic New Zealand rabbits. All stents had a diameter of 3.0mm and a length of 15mm. Group 1 (n=10), the control group, received bare stents, group 2 (n=10) received a PC-coated stent with low-dose irinotecan (46.5µg), and group 3 (n=10) received a PC-coated stent with high-dose irinotecan (1.29mg). All rabbits were sacrificed by the second month after stent-implantation. Morphometric analysis was performed to assess neointimal growth thickness (NT). A semi-quantitative injury score (from 0 to 3) was used to analyze necrosis (N), macrophages (M), edema (E), hemorrhage (H), and fibrin content (F), in the stented segments.
Results: In 3 cases the procedure was unsuccessful and 4 rabbits died before completing the 2-month follow-up period. These cases were excluded. No differences were observed in terms of injury score. The following table displays the study results.(*P< 0.05 vs Group 1).

	NT	N	M	E	H	F
Group1 (n=7)	207.9±31.6	0	1.14±0.15	0	0	0.50±0.20
Group2 (n=7)	156.3±36.5	0	0.75±0.29	0	0	0.75±0.29
Group3 (n=9)	113.4±17.2*	0	1.29±0.20	0	0	1.00±0.00*

Conclusions: PC-coated stents loaded with high-dose irinotecan inhibit neointimal thickening in the abdominal aorta of hypercholesterolemic rabbits. This effect was not accompanied by cellular injury.

1031-202 **Active Glycogen Synthase Kinase-3 Gene Transfer Inhibits Smooth Muscle Proliferation and Neointima Formation After Balloon Injury in Rats**

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Background: Glycogen Synthase Kinase(GSK)-3 is a crucial factor in many cellular signalling pathways and plays an important role in protein synthesis, cell proliferation, differentiation, microtubule dynamics, cell motility, and survival. We recently reported the antiproliferative and antimigratory effects of active GSK-3β gene transfer in human aortic smooth muscle cells in vitro.
Methods: To investigate the effect of active GSK-3β gene transfer on the formation of neointima after balloon injury in rats in vivo, we delivered adenoviral vectors expressing the constitutively active form of GSK-3β (GSK-S9A: 9th serine switched to alanine) or a control gene into rat carotid arterial segments after balloon injury with a 2F Forgarty catheter. Viral infusion mixtures containing 5x10^8pfu of virus diluted to a total volume of 100uL were incubated in the arterial lumen for 20minutes and the effects of gene delivery were evaluated 3 days and 2 weeks after gene delivery with morphometry and immunohistochemical staining.
Results: There were no significant differences in intimal, medial, and lumen areas at 3 days after the procedure. However, two weeks after gene delivery, the active GSK-3β gene transfer resulted in a significantly lower intima/media (I/M) ratio (0.29±0.06 vs 0.86±0.09, p<0.01) and a greater lumen area (0.41±0.02 vs 0.31±0.01mm^2, p<0.01) compared to the control gene transfected group. This was due to a significant reduction in intimal area (0.05±0.01 vs 0.15±0.02mm^2, p<0.01) while the medial area was similar (0.17±0.01 vs 0.18±0.01 mm^2, p=0.21). Proliferating cell nuclear antigen (PCNA) positive cells were significantly reduced in the neointima at 14 days in the active GSK-3β gene transferred group (2.97±0.29 vs 5.71±0.50%, p<0.01).
Conclusion: In vivo delivery of the active GSK-3β gene inhibits smooth muscle proliferation and neointima formation after balloon injury in rats, and may be a future target for gene therapy to inhibit restenosis after balloon angioplasty.

POSTER SESSION

1051 Pharmacology and Intervention

Sunday, March 30, 2003, 3:00 p.m.-5:00 p.m.
McCormick Place, Hall A
Presentation Hour: 3:00 p.m.-4:00 p.m.

1051-187 **The Smoker's Paradox in Patients Undergoing Percutaneous Coronary Intervention: A Report From the Dynamic Registry**

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Background: Despite the known detrimental effects of smoking on the incidence of and mortality from CAD, a paradoxical beneficial effect has been observed in smokers with myocardial infarction (MI). Methods: To determine whether the smoker's paradox exists for patients undergoing percutaneous coronary intervention (PCI), we evaluated 4,369 patients in the 1997-99 NHLBI Dynamic Registries, of whom 1,138 were current (CSM), 1,762 former (FSM) and 1,469 never (NSM) smokers. All comparisons were made to the NSM group. Results: CSM and FSM patients were significantly (all p<0.001) younger (55.9 vs 64.4 vs 65.7 yrs) and less often female (31.7 vs 26.3 vs 50.0%) in comparison to NSM. Among CSM, the prevalence of diabetes (20.2 vs 33.7%), hypertension (53.7 vs 66.0%), CHF (5.6 vs 11.3%), prior PCI (20.5 vs 29.1%), and prior CABG (7.6 vs 17.4%) was lower whereas the prevalence of pulmonary disease (10.3 vs 4.0%) and presentation with an MI (33.5 vs 20.5%) was higher compared to NSM (all p<0.001). Angiographic differences also existed, with CSM significantly (all p<0.001) more likely to present with single-vessel disease (47.9 vs 42.2%), lesion location in the right coronary artery (36.7 vs 30.5%), and to have vessels that were more thrombotic (24.7 vs 16.3%), ulcerated (13.9 vs 10.5%), occluded (17.3 vs 11.6%), and collateralized (16.0 vs 12.4%) in comparison to NSM. Despite similar procedural success rates, in-hospital mortality was significantly lower in CSM and FSM (0.8 and 1.0 vs 2.0%, both p<0.05) than in NSM. However, after adjustment for baseline differences, the risk of mortality did not differ from NSM (CSM vs NSM Odds Ratio 0.58, 95% CI 0.24, 1.39 and FSM vs NSM Odds Ratio 0.61, 95% CI 0.31, 1.19). At one year, mortality (3.7 vs 5.7%) and repeat revascularization (16.5 vs 20.6%) rates were significantly (p<0.05) lower in CSM compared to NSM patients but again, after adjustment, the relative risk of death, MI or repeat revascularization was sim-