lar smooth muscle cells (SMC). Retinoids have been shown to inhibit cellular proliferation possibly due to effects on cytokines, cytokine receptors and proto-oncogene expression.

Objective: In this study we examined whether all trans retinoic acid (RA) or its derivatives 13-Cis RA and 9-Cis RA would prevent the 5HT induced SMC proliferation as determined by ^{3}[H]-thymidine incorporation into DNA.

Methods: Canine aortic primary SMC (in 2nd or 3rd passage) were incubated with different concentration of RA, 13-Cis RA or 9-Cis RA and 200μM of serotonin for 20 hours in serum free medium. Then ^{3}[H]-thymidine (1μCi/plate) was added and ^{3}[H]-thymidine incorporation into SMC was measured 4 hours later.

Results: The 200μM of serotonin induced approximately a 3–4 fold increase in ^{3}[H]-thymidine incorporation (5570 ± 242 cpm/10^6 cells to 18202 ± 881 cpm/10^6 cells) in SMC (n = 5). All three retinoids inhibited serotonin induced SMC proliferation. The dose of retinoids that produced a 50% reduction in DNA ($p < 0.0001$) synthesis in SMC were 47nM for RA, 410nM for 9-Cis RA, and 5μM for 13-Cis RA. There was no evidence of cytotoxicity with any of the retinoids to SMC up to a concentration of 1–5 mM.

Conclusion: Retinoids prevent 5HT induced SMC proliferation at low concentrations. Since 5HT released from platelets at sites of vascular injury may play a role in the development of neointimal proliferation following coronary angioplasty, retinoids may potentially be useful for prevention of restenosis following angioplasty.

2:30

713-3 β-Blocker Agents Prevent Oxygen Radical-induced Human Apolipoprotein-B_{100} Oxidation

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Oxygen radicals may induce both peroxidation of polyunsaturated lipids and protein alterations in low density lipoprotein (LDL). Oxidized LDL taken up by macrophages via the "scavenger" receptor may contribute to formation of foam cells. Previous studies have shown that β-blocker agents reduce experimental atherosclerosis in primates. In addition, we have previously shown that β-blocker agents inhibit oxygen radical-induced lipid peroxidation. Thus, in this study we have investigated whether β-blockers may prevent oxidative modifications induced by oxygen radicals on apolipoprotein-B_{100} (apo-B_{100}) of LDL. Purified human LDL was exposed to oxygen radicals generated by $CuSO_4$ (15 μM for 20 hs at 37° C) under control conditions, or after a 30 min pre-incubation with propranolol (P; 1–10 mM). The index of peroxidation malonildihaldehyde, by thiobarbiturate method, was 2.1 ± 0.5* nmoles/mg of protein in native LDL, 34.5 ± 4.1 in control LDL, and decreased to 6.5 ± 1.8* in LDL incubated with P (*$p < 0.01$ vs controls). When oxidized LDL was run on SDS-polyacrylamide electrophoresis (SDS-PAGE; 5 to 10% gradient) extensive apo-B_{100} fragmentation was observed. Addition of P prevented the apo-B_{100} fragmentation (mean reduction from 12 to 3 bands of low molecular weight). Moreover, oxidized LDL had increased mobility on 0.8% agarose gel electrophoresis (2.8 ± 0.4 time than controls). Similarly, incubation with P reduced LDL mobility to 0.8 ± 0.2 fold in respect to controls ($p < 0.05$).

These data demostrate protection by propranonol on apo-B_{100} oxidation in vitro, at clinically relevant concentrations. Although β-blockers are known to adversely affect lipid metabolism, inhibition of both LDL peroxidation and apo-B_{100} fragmentation may reduce uptake of oxidized LDL by macrophages and hence foam cell formation.

2:45

713-4 Inhibition of Vascular Superoxide Production in Hypercholesterolemic Rabbit Aorta by L-Arginine Contributes to Restored Endothelium-dependent Relaxation

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Chronic oral administration of L-arginine (L-ARG) has been shown to enhance endothelial function in cholesterol (CHOL)-fed rabbits and to reduce atherogenesis. We investigated whether modulation of endogenous NO production (as assessed by urinary NO_3^- excretion) by L-ARG and the inhibitor of NO synthesis, L-NAME, affects vascular superoxide (O_2^-) production in hypercholesterolemic rabbits. Phorbol-myristate-acetate (PMA)-stimulated O_2^- production from isolated aortic rings was increased in rabbits given CHOL (+159 ± 28%) or CHOL + L-NAME (+149 ± 37%) as compared to controls (−22 ± 7%), and endothelium-dependent relaxations by acetylcholine were diminished in both groups. In aortic rings from rabbits given CHOL + L-ARG, PMA-induced O_2^- production was restored to control levels (+14 ± 17%; $p < 0.05$), and endothelium-dependent cholinergic relaxations were also partly restored. Urinary NO_3^- excretion decreased in all animals fed a CHOL-enriched diet ($p < 0.01$). As NO inactivated by O_2^- is also oxidized to NO_3^-, this indicates a decreased endothelial production of NO. NO_3^- excretion was further decreased by L-NAME ($p < 0.05$ vs. CHOL), and partly restored by L-ARG ($p < 0.05$). We conclude that both a decreased production of NO and an enhanced breakdown of NO by O_2^- contribute to the diminished biological activity of endothelial NO in hypercholesterolemia. L-ARG restores endothelial function by enhancing NO formation and by protecting NO from early breakdown by O_2^-.

3:00

713-5 N^{ω}-nitro-L-arginine Methyl Ester Increases Mural Platelet Deposition and Neutrophil-endothelial Interactions Under Low and High Shear Conditions

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Whether infusion of a nitric oxide (NO) synthesis inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME), can modulate mural platelet deposition and neutrophil interaction with the endothelium under flow conditions was examined in ex vivo bioassay experiments. Porcine aortic media, simulating deep arterial wall injury, or porcine aortic endothelium was exposed to flowing arterial blood from L-NAME-treated pigs for 5 min at 37°C under low (424 sec^{-1}) and high (3397 sec^{-1}) shear conditions in superfusion flow chambers. Intravenous administration of L-NAME (3 mg/kg bolus + 3 mg/kg/h perfusion) increased systolic (from 82.2 ± 2.7 to 108.5 ± 3.8 mmHg; $p < 0.001$), diastolic (from 47.4 ± 2.1 to 76.6 ± 3.6 mmHg; $p < 0.001$) and mean (from 59.0 ± 2.2 to 87.2 ± 3.6 mmHg; $p < 0.001$) arterial blood pressures with a slight reduction in heart rate (from 155 ± 8 to 139 ± 7 beats/min; $p < 0.01$). ^{51}Cr platelet deposition on aortic media exposed to low and high shear rates was increased, respectively, from 15.9 ± 2.9 to 20.4 ± 2.8 × 10^6/cm^2 ($p < 0.05$) and from 71.4 ± 11.9 to 95.8 ± 12.5 × 10^6/cm^2 ($p < 0.02$) following L-NAME treatment. ^{111}In neutrophil adhesion to intact endothelium was also enhanced by L-NAME from 9.8 ± 2.4 to 18.3 ± 5.2 × 10^3/cm^2 ($p < 0.03$) and from 12.5 ± 4.1 to 29.1 ± 8.8 × 10^3/cm^2 ($p < 0.02$) under respectively low and high shear rates. However, platelet interaction with the endothelium remained low and the endothelium maintained its thromboresistance.

Conclusion: Administration of the specific inhibitor of NO synthesis L-NAME increased arterial blood pressure and enhanced platelet interaction with the injured vessel wall and neutrophil adhesion to thromboresistant endothelium under low and high shear conditions.

3:15

713-6 Functional and Metabolic Protection of the Ischemic Rabbit Heart by NO Synthase Inhibitor

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To assess the role of the NO pathway in ischemic injury, the NO synthase inhibitor L-N-Monomethylarginine (L-NMMA) was tested on isolated rabbit hearts retrogradely perfused with Krebs-Henseleit buffer containing 5.5 mM glucose and submitted to 30 min equilibration at 5 ml/min per g, followed by a 60 min low-flow ischemia (10% baseline) and 30 min reperfusion. Metabolic and physiological parameters of hearts treated with 1 μM L-NMMA added 15 min before ischemia (n = 10) were compared with similar parameters in controls (ctrl, n = 15) without the inhibitor. Before ischemia, L-NMMA did not influence left ventricular developed pressure or coronary perfusion pressure but lowered cyclic GMP by 25% ($P = 0.01$). During ischemia, L-NMMA decreased the severity of ischemic contracture (25 ± 3 vs 54 ± 4 mmHg in ctrl, $P < 0.001$). Perfusion with D-NMMA did not protect the heart against ischemic contracture. The effect of L-NMMA was suppressed by 1 mM L-arginine or 10 μM sodium nitroprussiate but not by 10 μM 8-Br-cyclic GMP, a cyclic GMP analogue. At the end of ischemia, exogenous glucose uptake was 0.90 ± 0.08 and 4.0 ± 0.2 μmol/min per g DW ($P < 0.01$), lactate production was 4.0 ± 0.7 and 8.0 ± 0.7 μmol/min per g DW ($P < 0.01$), glycogen was 15 ± 2 and 29 ± 5 μmol glucose eq./g DW ($P < 0.01$) and ATP was 2.5 ± 0.4 and 4.6 ± 0.3 μmol/g DW ($P < 0.01$) in ctrl and treated hearts, respectively. With reperfusion, the developed pressure was doubled (65 ± 5 vs 32 ± 3 mmHg, $P < 0.001$) and the CPK release reduced by 50% ($P < 0.01$) in hearts treated with L-NMMA vs ctrl. A dose-response curve of protection against ischemic damage by L-NMMA showed that the NO synthase inhibitor decreased ischemic contracture and enhanced glucose uptake between 1 nM and 1 μM, i.e. below concentrations inducing vasoconstriction. It is concluded that L-NMMA protects the ischemic rabbit heart against cellular damage by stimulating exogenous glucose uptake, thereby preserving endogenous energetic resources as glycogen. This protection during ischemia leads to an improvement of post-ischemic functional recovery. This effect during ischemia seems cGMP-independent.

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