and IGF-1. RV mRNA levels were linearly related (p<0.01) with those of the LV, both for ACE (r=+0.88) and ET-1 (r=+0.71).
The present study showed that LV dysfunction in a model of selective RV overload is accompanied by biventricular activation of regulatory (ACE and ET-1) systems, while counter-regulatory BNP is selectively activated in the RV. These findings might add to the understanding of the relative importance of load and autocrine/paracrine activation in the progression to heart failure.

1029-184 The Clinical Significance of a Common, Functional, X-Linked Angiotensin II Type 2-Receptor Gene Polymorphism (-1332 G/A) in a Cohort of 509 Families With Premature Coronary Artery Disease

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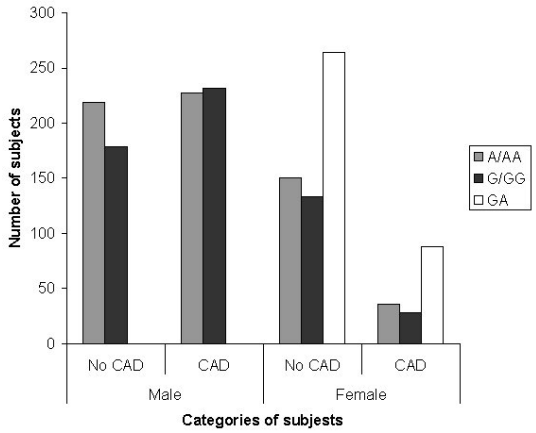
Background: A common intronic polymorphism, (-1332 G/A) of the angiotensin type 2 (AT_2) receptor gene, located on the X-chromosome, has been reported to be biochemically functional.
Methods: We investigated 509 families from a cohort of families with a history of premature coronary artery disease (CAD). Families consisted of one sibling affected with premature CAD and two unaffected siblings. Genotyping of subjects was performed using a restriction enzyme digestion of an initial 310 bp PCR fragment that included the AT_2 (-1332 G/A) locus.
Results: The mean age of the 611 siblings affected by premature CAD at the time of event was 49.5 ± 8.1 yrs. Conditional logistic regression analysis confirmed a significant predictive value of premature CAD for the covariates of hypertension, diabetes, dyslipidaemia, history of smoking, as well as male gender (p<0.005). The genetic data were analysed using the XS-TDT statistics program which allows the calculation of exact p-values where the null hypothesis is of no linkage. In men (but not women) the G allele occured significantly more frequently than would be expected if the disease causing locus and AT_2 (-1332 G/A) locus were unlinked; p-exact value = 0.024. The data were further analysed to investigate linkage between this locus and a causative locus for hypertension. In men we observed a trend towards linkage; p-exact value = 0.08.
Conclusion: We have found evidence of linkage between the AT_2 (-1332 G/A) locus, and a causative locus for premature CAD in men but not in women.



1029-185 Mobilization of Bone Marrow Cells With Granulocyte-Colony Stimulating Factor Increases the CD34-Positive Mononuclear Cells in Peripheral Blood and Improves Angiogenesis, Ventricular Remodeling and Heart Function After Acute Myocardial Infarction in Rats

Hong Liu, Jianhui Shi, Naisheng Cai, Junbo Ge, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China

Background: It's reported that bone marrow endothelial progenitor cells (EPCs) can be mobilized by myocardial infarction (MI). The aim of this study was to investigate the effect of granulocyte-colony stimulating factor (G-CSF) in mobilizing the putative precursor of EPCs [CD34-positive monoculear cells ($MNCs^{CD34+}$)] from bone marrow and the correlation to heart function in a rat MI model. **Methods**: G-CSF (n=8, 150 μg/kg/day) or placebo (n=8) were given subcutaneously to left coronary artery-ligated rats for 5 days after operation. On the 6th post-operation day, MNCs were seperated and labeled with anti-CD34 for FACS analysis. Echocardiography was performed for assessment of heart function before rats were killed at 6 weeks after operation. **Results:** 1) Leucocytes and MNCs in peripheral blood in CSF-treated group were increased by 2-fold (25.17±10.93/ml vs. 10.14±3.09/ml* and 20.55±10.42/ml vs. 9.12±2.86/ml*, *p<0.05 vs. G-CSF group), neutrophil by 4-fold (4.61±2.95/ml vs. 1.02±0.6/ml*), $MNCs^{CD34+}$ by 6-fold (262.79±73.63/μl vs. 43.9±29.77/μl*) compared to the control group 6 days after MI. Erythroctyes and thrombocytes were similar between the two groups. 2) The capillary density of the CSF-group in scar and border zone was increased significantly. 3) LVDD (left ventricular end-diastolic diameter, 0.83±0.09 cm vs. 0.70±0.08 cm*) was attenuated, scar thickness increased (0.13±0.02 cm vs. 0.17±0.02 cm*) and LVEF increased (88.89±4.14% vs. 66.22±13.35%*) by G-CSF compared to the control group 6 weeks after MI; 4) The number of circulating $MNCs^{CD34+}$ was positively correlated with LVEF (r=0.84, p<0.05). **Conclustions**: Mobilization of bone marrow cells with G-CSF increases the CD34-positive mononuclear cells in peripheral blood and improves angiogenesis, ventricular remodeling and heart function after acute myocardial infarction in rats.

POSTER SESSION

1045

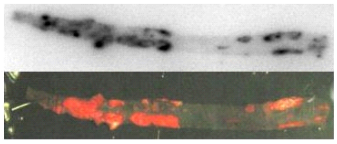
Therapeutic Angiogenesis From Bench to Bedside

Sunday, March 07, 2004, 3:00 p.m.-5:00 p.m.
Morial Convention Center, Hall G
Presentation Hour: 3:00 p.m.-4:00 p.m.

1045-189 Plaque Targeting in Atherosclerotic Mice Using a Small ImmunoProtein Against an Angiogenesis-Associated Fibronectin Isoform

Pia K. Schuler, Christian M. Matter, Patricia Meier, Romeo Ricci, Dongming Zhang, Patrizia Alessi, Dario Neri, Thomas F. Lüscher, University of Zurich, Zurich, Switzerland, Federal Institue of Technology, Zurich, Switzerland

Background: Currently, human atherosclerotic plaques can be visualized by angiography, ultrasound, CT and MRI. However, these imaging modalities are restricted to detect advanced atherosclerotic lesions. The aim of our study was to visualize early atherosclerotic plaques at the molecular level using an antibody against the extra-domain B of fibronectin (L19).
Methods: L19 was marked with radioactivity (I-125) or fluorescence (Cy5) and injected intravenously in apolipoprotein E knockout (ApoE ko) and corresponding control mice. 24h after injection of the tagged L19, the aorta was isolated for en face and cross sectional analysis. An unspecific antibody was used as a control for L19.
Results: Plaques could be clearly visualized in all ApoE ko mice after injection of labeled L19 (Fig.A). Specifically, the signal after phosphorimaging of the en face aorta following injection with radiolabeled L19 exactly matched the fat staining of plaques using Oil Red O (Fig.B). Similarly, fluorescent staining with Cy5 corresponded with plaques on cross sections. No signal was observed in normal vessels and in ApoE ko mice injected with the unspecific antibody.
Conclusions: L19 enabled us to specifically identify atherosclerotic plaques and thus, may provide a novel molecular tool for plaque diagnosis. Furthermore, these findings may set the stage for a non-invasive plaque therapy by coupling anti-atherogenic drugs to the proposed antibody.



Specific plaque imaging in ApoE ko mice using L19. En face phosphorimaging following injection with radiolabeled L19 (A) matches atherosclerotic plaques visualized by fat staining (B).

1045-190 Documentation of Exercise-Induced Coronary Collateral Growth in Humans

Rainer Zbinden, Stefan Zbinden, Kerstin Wustmann, Michael Billinger, Jean-Paul Schmid, Stefan Windecker, Bernhard Meier, Christian Seiler, Cardiovascular Center Bern, Bern, Switzerland

Background: Collateral vessels can reduce the often fatal consequences of a sudden coronary artery occlusion. Collateral arteries develop out of preexesting arteriolar anastomoses in response to increased shear forces. We hypothesized that a long-term exercise program (increasing vascular shear forces) leads to:
1)Increased collateral flow as obtained in an angiographically normal coronary artery
2)Lack of decrease in collateral flow expected after percutaneous coronary intervention (PCI) in a diseased coronary artery
3) A dose-response relation between the degree of fitness following training and change in coronary collateral flow.
Methods: Thirty-four patients (age 61 ± 8 years) undergoing PCI because of stable angina pectoris were included in the study. They performed a 3-months physical endurance exercise program (3 times per week, 1 hour per session) with baseline and follow-up invasive collateral flow index measurements (CFI, no unit). CFI was measured using a sensor-tipped PCI guidewire distal to the balloon-occluded coronary artery in a vessel without stenosis and in a diseased coronary artery undergoing ad-hoc PCI. Spiroergometry was performed at baseline and follow-up.
Results: There was a significant increase in CFI in the angiograpically normal vessel (baseline: 0.19 ± 0.09; follow-up: 0.23 ± 0.08; p=0.003) and the expected decrease in CFI in the vessel undergoing PCI was prevented (baseline: 0.18 ± 0.09; follow-up: 0.20 ± 0.06; p=NS). There was a direct dose-response relation between delta VO2max and change of CFI in the normal vessel (p=0.04;r= 0.42).
Conclusion: Endurance exercise training induces the growth of collateral arteries ("arteriogenesis") in a myocardial region supplied by an angiographically normal vessel and it

prevents the expected reduction in collateral flow after PCI of a stenotic vessel. There is a direct dose-response relation between exercise capacity gained and coronary collateral flow augmentation in the angiographically normal vessel.

1045-191 High-Density Lipoprotein-Induced Tube Formation Requires the Activation of Ras/Raf/Mitogen-Activated Protein Kinase in Human Coronary Artery Endothelial Cells

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Objectives: High density lipoprotein (HDL) levels have been shown to be inversely correlated with coronary artery disease, but the mechanisms of the direct protective effect of HDL on endothelial cells (ECs) are not fully understood. In this study, we investigated the role of the HDL–mediated promotion of angiogenesis in human coronary artery ECs (HCECs).
Methods and Results: We developed an in vitro model of HCEC tube formation on a matrix gel. HDL induced tube formation, in which the dose-response showed that the maximum effective dose of HDL was 100 μg/ml. We also examined the effect of sphingosine-1-phosphate (S1P), which is a carrier of bioactive lipids of HDL, to analyze tube formation. Since HDL contains S1P (about 180 pmol/mg protein), 100 μg/ml of HDL contains about 0.018 μM of S1P. Although we observed 0.02 μM of S1P significantly induced tube formation, it was only 20 % of 100 μg/ml HDL-induced tube formation. HDL-induced tube formation may be partly mediated by S1P. PD98059, an inhibitor of p42/44 mitogen-activated protein kinase (MAPK) activity, but not SB203580, an inhibitor of p38 MAPK activity, suppressed HDL-induced tube formation. Dominant-negative Ras N17 inhibited HDL-induced tube formation. HDL activated Ras by ras pull-down assay and its effect was inhibited by pertusis toxin (PTX). Moreover, HDL activated phospho(p)-p42/44 MAPK, while Ras N17 blocked HDL-induced pp42/44 MAPK.
Conclusions: These results indicate that HDL induced a potent signal through a Ras/MAPK pathway mediated by PTX-sensitive G-protein coupled receptor to the angiogenic phenotype in HCECs.

1045-192 Monocytes, but Not Neutrophils or Lymphocytes Are Essential Mediators of Arteriogenesis

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Background: Blood vessel growth following arterial occlusion is mediated by infiltrating leukocytes. While all three leukocyte subpopulations (neutrophils, lymphocytes, monocytes) are known to play an important role in angiogenesis, only monocytes have been shown to influence the growth of collateral arteries (arteriogenesis). In this study we examined the importance of neutrophils and lymphocytes in a rabbit hindlimb model of arteriogenesis. **Methods:** 36 Rabbits received either Interleukin-8 (IL-8), Neutrophil-activating-protein-2 (NAP-2) or Lymphotactin (Ltn) via osmotic minipumps directly into the collateral circulation after femoral artery ligation. NAP-2 is a relatively selective activator of neutrophils, Ltn chemoattracts lymphocytes and Il-8 has an effect on both celltypes. PBS and MCP-1 treated groups served as controls. After one week, leukocytes around growing collateral arteries were quantified via immunohistology and effects on integrin markers of leukocytes activation (Mac-1, LFA-1) were assessed by flow-cytometry. Collateral conductance was assessed using fluorescent microspheres. **Results**: Although a significant increase in neutrophil accumulation after IL-8 and NAP-2 treatment was detected in-vivo (cells/mm^2: PBS:8,33±2,87,MCP-1:25,23±10,81,IL-8:36,06±12,65,NAP-2:20,67±7,41,Ltn:8,27±5,44) and Il-8 and NAP-2 resulted in neutrophil activation in flow-cytometry (Mac-1 Expression: PBS:21,12±2,11,MCP-1:24,11±2,64,IL-8:32,65±2,10,NAP-2:27,30±2,25, Ltn:17,30±2,16), no significant increase in collateral conductance was observed. Ltn treatment resulted in lymphocyte accumulation (cells/mm^2: PBS:2,48±1,24,MCP-1:4,26±1,16,IL-8:6,88±5,28,NAP-2:2,70±0,57,Ltn:12,80±3,50), but not in collateral artery growth. Collateral conductance: [ml/min/100mmHg]: PBS:50,70±5,15, MCP-1:339,60±19,6, IL-8:58,91±5,56, NAP-2:66,83±8,72, Ltn:52,80±5,37. **Conclusion:** While lymphocytes and neutrophils are known to participate in angiogenesis, their importance for arteriogenesis seems to be neglectible. These findings support the hypothesis of monocytes/macrophages as key mediators of collateral artery growth.

1045-193 Granulocyte-Colony Stimulating Factor Mobilizes and Activates Endothelial Progenitor Cells in Patients With Coronary Artery Disease

Tiffany M. Powell, Jonathan D. Paul, Jonathan M. Hill, Elizabeth J. Read, Philip J. McCoy, Richard O. Cannon, III, National Institutes of Health, Bethesda, MD

Background: Circulating endothelial progenitor cells may function to repair cardiovascular injury, but are reduced in patients with coronary artery disease. Granulocyte-colony stimulating factor (G-CSF) mobilizes hematopoietic stem cells (CD34+) in healthy subjects, but whether this cytokine mobilizes endothelial progenitor cells capable of endothelial maturation in coronary artery disease patients is unknown. **Methods:** G-CSF (10 mcg/kg) was administered daily for 5 days to 12 coronary artery disease patients, and circulating CD34+ cells, endothelial progenitor cells (CD133/ VEGFR-2+), and mature endothelial cells [CD144 (VE-cadherin), CD31 (PECAM), CD51/61 (alpha$_v$beta$_{III}$ integrin)] were measured by flow cytometry. **Results:** G-CSF increased CD34+ cells from <1 cell/microL of blood at baseline to 60±18 cells/microL within 24 hours of the last dose (p<0.001), similar to CD34+ mobilization achieved in 28 healthy donors >40 years of age at our hospital (78±8 cells/microL). Endothelial progenitor cells were also mobilized, although to low levels (from <1 cell/microL at baseline to 6±3 cells/microL within 24 hours of last G-CSF dose, p<0.001). Mature circulating endothelial cells also increased post-G-CSF: CD34/CD144+ from 16±16 to 107±90 cells/microL, CD34/CD31+ from 54±28 to 224±60 cells/microL, CD34/CD51/61 from 14±14 to 81±64 cells/microL (all p<0.05 vs baseline). One week following completion of treatment, CD34+ cells and endothelial progenitor cells had returned to baseline, but levels of mature endothelial cells remained increased over baseline (p<0.05). **Conclusion:** These findings establish that G-CSF administration to coronary artery disease patients mobilizes CD34+ cells, which includes the endothelial progenitor cell subset, into the circulation. Mobilization is associated with increased cells expressing mature endothelial markers, which persist even at one week following the last dose of G-CSF. This suggests sustained G-CSF-stimulated differentiation along an endothelial cell lineage, with potential therapeutic implications for neovascularization of ischemic myocardium.

1045-194 Impaired Arteriogenic Response to Acute Hindlimb Ischemia in CD8 Knockout Mice

Eugenio Stabile, Mary Susan Burnett, Timothy D. Kinnaird, Craig Watkins, Cheol W. Lee, Matie Shou, Andrea la Sala, Schmuel Fuchs, Stephen E. Epstein, Washington Hospital Center, Washington, DC

Background: CD8$^+$ cytotoxic T lymphocytes regulate cellular responses of the immune system, which play a pivotal role in modulating collateral vessel development.
The aim of our study was to investigate if the absence of circulating CD8$^+$ T-cells impairs collateral development after femoral artery ligation in CD8 -/- mice.
Methods and Results: After surgical excision of the femoral artery, Laser Doppler Perfusion Imaging demonstrated reduced collateral flow induction in CD8 -/- mice compared to control mice (C57BL/6J) at day 3 (0.21±0.01 vs 0.29±0.03, p< 0.01) which persisted to day 28 (0.69±0.04 vs 0.90±0.04, p< 0.01). In CD8 -/- mice , when compared to controls, the biological importance of the reduced collateral flow was evident by diminished recovery of hindlimb function (ambulatory impairment score: 1.73 ± 0.18 vs 0.86 ± 0.19, p< 0.01), greater calf muscle atrophy (mean fiber area 767 ± 68 vs 1067 ± 69, μm^2, p< 0.01), and increased fibrotic tissue content (14 ± 1% vs 7 ± 1%, p< 0.01). Exogenous CD8$^+$ T-cells, when infused into CD8-/- mice immediately after ischemia induction, selectively home at the site of collateral formation and, over time, significantly increased collateral flow, improved hindlimb functional recovery, and reduced muscle atrophy/fibrosis.
Conclusions: These results demonstrate that CD8+ T-cells are a critical component of the immune system in regulating the early phase of normal collateral development. CD8 -/- mice demonstrated both delayed and impaired blood flow recovery after femoral artery ligation, and infusion of CD8$^+$ T cells immediately after surgery rescued the phenotype. Our study provides further evidence that the immune system is critical in modulating collateral development in response to peripheral ischemia.

1045-195 Monocyte Chemoattractant Protein-1 Activates Vascular Endothelial Growth Factor- and Tumor Necrosis Factor-Alpha-Mediated Angiogenesis in Ischemic Hindlimbs of Mice

Hiroshi Niiyama, Hisashi Kai, Hironobu Tahara, Toyoaki Murohara, Tsutomu Imaizumi, Kurume Univeristy, Kurume, Japan

Recently, we and others have suggested that macrophage accumulation plays a role in angiogenesis in hindlimb ischemia model. Macrophage chemoattractant protein (MCP)-1 is a key molecule to trigger inflammatory changes in various diseases. Thus, we sought to determine the role of the endogenous MCP-1 in ischemia-induced angiogenesis. At day 0, unilateral hindlimb ischemia was induced by excising surgically entire left femoral artery and vein in mice. Immediately after operation, plasmid DNA encoding 7ND, a dominant negative mutant of MCP-1, or the empty plasmid (as control) was injected into the ipsilateral thigh adductor muscle. Serial laser Doppler blood flow analysis showed an abrupt decrease in blood flow, followed by a remarkable recovery, in ischemic hindlimbs of controls. Control mice showed well-developed collateral vessels and capillary formation as assessed by postmortem angiography and immunohistostaining for CD31, respectively, at day 21 after induction of ischemia. In 7ND-treated mice, although the extent of the early decrease in laser Doppler blood flow was similar to that in controls, the recovery was impaired. At day 3, macrophage infiltration and inductions of vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF)-alpha, known angiogenic factors, were prominent in the adductor muscle of ischemic hindlimbs in controls. 7ND treatment significantly reduced the number of infiltrated macrophages and repressed VEGF and TNF-alpha inductions in response to ischemia at day 3. Moreover, the number of angiographically visible collateral vessels and the capillary density were significantly decreased in ischemic hindlimbs of 7ND-treated mice at day 21. In conclusion, MCP-1-mediated macrophage accumulation may play an important role in ischemia-induced angiogenesis at least in part by activating induction of angiogenic factors such as VEGF and TNF-alpha in ischemic hindlimbs.

1045-196 Fiber Type-Specific Angiogenic Dysregulation in a Genetic Mouse Model of Heart Failure

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Introduction. Chronic heart failure (CHF) leads to intrinsic skeletal muscle abnormalities including slow-oxidative to fast-glycolytic fiber type switching, decreased capillary density, and reduced mitochondrial function. These skeletal muscle abnormalities contribute to clinical exercise intolerance. **Methods**. A genetic mouse model of heart failure induced through cardiac-targeted overexpression of the sarcoplasmic reticulum Ca^{2+} storage protein calsequestrin (CSQ) has been recently characterized. Skeletal muscle (plantaris) from CSQ/CHF mice and wild type (WT) mice was analyzed with triple color immunofluorescence using antibodies specific for myosin heavy chain I, IIa, IIb, and endothelial cells. **Results.** A decrease in oxidative myofibers (I + IIa), a concurrent increase in glyco-