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1.1 NADPH OXIDASES AS MAJOR SOURCE OF OXIDATIVE STRESS IN CARDIOVASCULAR DISEASE: DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

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Oxidative stress has been suggested to be a key pathomechanism of hypertension and ischemia-reperfusion injury. However, all therapeutic attempts to exploit this with antioxidants have been clinical failures. Here we present an alternative approach by identifying a relevant source of oxidative stress and its role in cardiovascular disease. NADPH oxidases (NOX) NOX are the only known enzymes with reactive oxygen species (ROS) as their sole product. In the vasculature, NOX1 may contribute to endothelial dysfunction by scavenging NO and its anti-hypertensive and anti-atherosclerotic effects. Aged spontaneously hypertensive rats (SHR) develop endothelial dysfunction and increased ROS compared to aged matched WKY. This was inhibited by a NOX inhibitor. In contrast, eNOS or xanthine oxidase inhibition did not reduce ROS levels. NOX1 and NOX2 were upregulated in SHR aortae compared to WKY rat aortae, whereas NOX2 and 4 expression remained unchanged. Also, NOX4 knockout mice had normal basal blood pressure. NOX1 showed strong positive staining in the intima of SHR, where it co-localized with an endothelial cell marker. Aortic endothelial function was significantly impaired in SHR versus WKY rats. The NADPH oxidase inhibition improved aortic relaxation more pronounced in SHR. In conclusion, ROS formation and NOX1/2 expression are increased in a NOX inhibitor-reversible manner. NOX1 may thus represent a novel target for the treatment of hypertension. Conversely, NOX4 is induced in hypoxia/ischemia and essential for the subsequent reperfusion injury in stroke. Thus NOX1 and NOX4 represent novel cardiovascular targets for specific treatment of pathological oxidative stress.

1.2 DIRECT AT2-RECEPTOR STIMULATION IMPROVES SURVIVAL AND NEUROLOGICAL OUTCOME AFTER EXPERIMENTAL STROKE (MCAO) IN MICE

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This study investigated the effects of specific and selective AT2-R stimulation on infarct size, survival and neurological outcome after middle cerebral artery occlusion (MCAO) in mice and underlying molecular mechanisms. C57BL-6 or AT2R-deficient mice (on C57BL-6 background) underwent MCAO for 30 minutes followed by reperfusion. Mice were treated daily with either vehicle (0.9% NaCl i.p.) or Compound 21 (C21) (0.03 mg/kg i.p.) for 4 days starting 45 minutes after MCAO. Garcia neurological score was obtained to assess the severity of neurological deficits. Infarct volume was measured in vivo 96h post-stroke by MRI. Neurotrophin BDNF mRNA-levels and IL-6 mRNA-levels were measured in brain samples by quantitative RT-PCR. AT2R stimulation of wildtype mice did not significantly reduce infarct size compared to vehicle group. However, infarct sizes were larger in vehicle-treated AT2R-deficient mice compared to wildtype mice. Neurological deficits were significantly attenuated in C21-treated mice but unchanged in vehicle-treated mice. Mortality in vehicle-treated mice amounted to 57% and was significantly lowered to 28% in C21-treated mice. In contrast, there were no effects of C21 on neurological outcome and survival in AT2R-deficient mice. Gene expression of neurotrophin BDNF was strongly upregulated and IL-6 significantly downregulated in the infarcted brain areas of C21-treated mice compared to the vehicle group, while there were no changes in AT2R-deficient mice. C21 had no effect on blood pressure. Our data demonstrate for the first time that direct AT2R-stimulation by C21 improves survival and neurological deficits after experimental stroke through neuroprotective and anti-inflammatory, blood-pressure independent mechanisms, and these effects are AT2R-specific.

1.3 TREATMENT WITH THE MEK1/2 INHIBITOR U0126 ABOLISHES VASOCONSTRICCTOR RECEPTOR UPRREGULATION IN CEREBRAL ARTERIES AND IMPROVES NEUROLOGICAL OUTCOME AFTER SUBARACHNOID HAEMORRHAGE IN THE RAT

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Delayed cerebral ischemia caused by pathological cerebral vasconstriction is a cause of poor prognosis after subarachnoid haemorrhage (SAH). After SAH, cerebrovascular vasconstrictor receptors are upregulated by a process involving MEK-ERK1/2 signalling. We here investigated the effect of the MEK1/2 inhibitor U0126 on cerebrovascular receptor upregulation and delayed ischemia after SAH. SAH was induced in rats by injection of autologous blood into the basal cisterns. U0126 or vehicle was administered intracranially at 6, 12, 24 and 36 hours after SAH. Smooth muscle ETa and 5-HT1B receptors in cerebral arteries were studied by immunohistochemistry and myograpnic contractions studies. Neurological deficits were assessed by a rotating pole test. SAH enhanced endothelin-1 (ET-1)- and 5-carboxyamidotryptamine (5CT)-induced contractile responses of middle cerebral (MCA) and basilar (BA) arteries compared to sham-operated rats (in BA, respective 31.01 ± 6.43 Etα and 31.01 ± 8.47 5HT1B in SAH vs. 22.93 ± 20.3% and 169.23 ± 8.8%, respectively). Accordingly, SAH increased the protein expression of smooth muscle ETα and 5-HT1B receptors by 175.33 ± 13.77 and 167.72 ± 24.74%, respectively. In vivo treatment with U0126 completely abolished the SAH-induced increased arterial contractility and receptor expression. Rotating pole scores two days after surgery were 5.37 ± 0.23 in sham-operated rats, 3.35 ± 0.67 in SAH-induced rats and 5.00 ± 0.4 in SAH-induced U0126 treated rats. In conclusion, U0126 abolishes vasconstrictor receptor upregulation and alleviates neurological deficits after SAH, suggesting that the receptor upregulation contributes critically to delayed cerebral ischemia. MEK1/2 inhibition may represent a novel SAH treatment strategy.

1.4 DIRECT AND SELECTIVE AT2-RECEPTOR STIMULATION REDUCES LOCOMOTOR DEFICITS AND PROMOTES NEUROREGENERATION THROUGH INDUCTION OF NEUROTROPHINS IN AN ANIMAL MODEL OF SPINAL CORD INJURY

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This study aimed to test AT2R-stimulation by Compound 21 (C21) as a potential therapeutic approach for the treatment of experimental spinal cord injury in mice. To indentify underlying neuroprotective mechanisms complementary experiments in primary neurons and organotypic cultures were performed. Experimental spinal cord injury was induced by defined mechanical compression in Balb/C mice. Animals were treated with C21 (0.3 mg/kg/day i.p.) or vehicle for 4 weeks. For tracking of motor neurons fluorescent-labelled dextrane was injected into the left motor cortex. Locomotor deficits were evaluated by Basso Mouse Scale (BMS). Organotypic co-culture of GFP-positive entorhinal cortices with hippocampal target tissue served to evaluate the impact of C21 (1μM) on reinnervation. Neuronal differentiation (neurite outgrowth), sprouting (Gap-43), apoptosis (Bcl-2) and expression of neurotrophins (BDNF and BDNF-receptor TrkB) were investigated in primary murine, neuronal cells and partly in sections of spinal cords. C21 significantly reduced locomotor deficits and increased the number of motor neurons bridging the lesional area. C21 increased expression of TrkB in spinal cord and of Bcl-2 (+ 75.7%), BDNF (+ 53.7%), TrkB (+ 57.4%) and GAP43 (+ 103%) in primary neurons. C21 significantly induced reinnervation in organotypic culture (+ 50%) and neurite outgrowth (+ 25%) in primary neurons. C21-induced neurite outgrowth was abolished by co-culture with neurotrophin antagonists (anti-neraptin, anti-BDNF) and TrkB-deficient neurons. We conclude that AT2R-stimulation ameliorates locomotor deficits in experimen- tinal spinal cord injury through neuroprotective and neuroregenerative mechanisms such as increased BDNF/TrkB expression. Thus, AT2R-stimulation may be considered a novel therapeutic approach to promote and improve neuroregeneration.

1.5 CALCITONIN GENE-RELATED PEPTIDE (CGRP): AN ENDOSNEIOUS DOPING AGENT?

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Exhausting exercise strains the circulation enormously. With increasing intensity and duration of exercise cCGRP is released from the working muscles. Exercise performance correlates better with cCGRP plasma levels than with heart rate. The calcitonin receptor-like receptor / receptor activity modifying protein-1 (CLR/RAMP-1) complex forming functional cCGRP receptors was found on sympathetic neurons and stimulation of these receptors increases heart rate independent on blood pressure drop. We tested the hypothesis that cCGRP supports the circulation during exhausting exercise. Heart rate and blood pressure (using telemetry) as a function of exercise load and maximal oxygen consumption (VO2max) was measured in naive wild type (n-wt) mice, wt mice treated with the cCGRP antagonist CGRP8–37, wt-ag, mice lacking cCGRP (CGRP-ko) [Lu et al., 1998. Mol Cell Neurosci 14: 99–120]. Treatment of wt with CGRP8–37 reduced significantly VO2max by about 7%
which was identical to the drop of V02max found in CGRP-ko. In CLR-tg mice V02max was about 13% higher than in their wt controls. Whereas the heart rate was not significantly between the different experimental groups mean arterial blood pressure at the moment of V02max increased in the following order: CGRP-ko < wt-ag < n-wt < CLR-tg. Moreover, in comparison to wt-mice the heart index was about 10% higher in CLR-tg and about 10% lower in CGRP-ko mice. Our data clearly demonstrate that the level of CPG signaling modulates maximum exercise capacity.

2.1 BITOPIC AGONISM OF ENDOTHELINS AT ARTERIAL ETA-RECEPTORS

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Endothelin-1 (ET-1) causes long-lasting persistent contractions in arteries. We hypothesized that different parts of the agonist play different roles in initiation and maintenance of binding and activation of ETα-receptors. In isolated rat mesenteric resistance arteries, we recorded smooth muscle effects of putative ET-receptor ligands. 4N-Et-1 and ET-1 fragments ET-11-21, ET-11-15, and ET-11-21 (1 μM) did not display agonism or antagonism. ET-isofoms caused contractions (potency: ET-1 > ET-2 > ET-3), efficacy: ET-2 > ET-1 > ET-3. The ETα-antagonist BQ123 antagonised these effects with isofrom-dependent affinity (ET-3 > ET-1 > ET-2). It reduced contractions in the presence of an isofrom (R1) and contractions initiated by, and persisting in the absence of, a free isofrom (R0). The relaxation-amplitude differed from that predicted by the classical antagonism (A) and was dependent on the isofrom (ET-1: A = R0; ET-2: R1 + R2; ET-3: A = R1 + R2). The ETα-antagonist PD156707 competitively antagonised ET1-induced contractions and was less effective in relaxing than preventing contractile responses to ET-1 (A = R0). These observations indicate that ET-1, which cannot be subdivided into an address- and message-domain, binds to at least two sites on ETα-receptors (one is sensitive to low molecular weight antagonists, the other is characterized by slow complex-dissociation) that interact regarding ligand-binding affinity and receptor-activation. Thus, ET-1 is an endogenous bitopic agonist and modulating the alternative binding site(s) can be useful for pharmacotherapy of diseases involving this peptide.

This study was performed within the framework of Tl Pharma project T2–301.

2.2 MOLECULAR CHARACTERIZATION OF Ca2+-ACTIVATED Cl- CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS

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The presence of a Ca2+ -activated Cl- current in the vascular smooth muscle cells (VSMCs) is well established. This Cl- current has been suggested to be important for synchronized vasomotion (2 % vs. 16% of maximal tension). The frequency of oscillation is not affected (0.20 vs. 0.23 Hz). In conclusion, TMEM16A is expressed in VSMCs and is of vasomotion (2 % vs. 16% of maximal tension). The frequency of oscillation is not affected (0.20 vs. 0.23 Hz). In conclusion, TMEM16A is expressed in VSMCs and is

2.3 ENDOTHELIN-1, A KEY PLAYER DURING THE ONSET OF HYPERTENSION IN THE SPONTANEOUS HYPERTENSIVE RAT

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Introduction: Endothelin-1 (ET-1) a potent vasoconstrictor peptide is produced by Endothelin Converting Enzyme (ECF) and neutral endopeptidase (NEP). ET-1 is implicated in several predominantly salt-sensitive forms of experimental hypertension, but not in the spontaneous hypertensive rat (SHR). Hypothesis: ET-1 plays an important role during the onset of hypertension in SHR. Methods: The concentration of ET-1 in the left ventricle, kidney and lungs of 8, 6 and 12 weeks old SHR rats. Also, 4 weeks old SHR rats were treated during 4 weeks with the dual ECE/NEP inhibitor SOL-1 (50mg/kg d. s.c.). At 8 weeks of age intra arterial blood pressure was determined. Results: ET-1 protein levels are significantly increased in the left ventricle, kidney and lungs of versus 8 weeks old SHR. In 12 weeks old SHR ET-1 levels are only increased in the lungs compared to 6 weeks old SHR. In SOL-1 treated SHR (n–4) mean arterial pressure is significantly decreased as compared to vehicle treated SHR (n–14) (126 mmHg vs. 114 mmHg). Conclusion: During the development of hypertension but not during established hypertension ET-1 levels are increased in SHR. A pathogenic role of ET-1 is further supported by the blood pressure lowering effect of chronic ECE/NEP inhibition during the onset of hypertension.

This research was performed within the framework of projects T2–108 and T2–301 of the Dutch Top Institute Pharma.

2.4 TLR4-/−SHOWS ADVANTAGEOUS CHARACTERISTICS IN A MODEL OF L-NAME-INDUCED HYPERTENSION

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Background: Hypertension-induced cardiovascular disease is a leading cause of mortality. Innate immunity, in particular Toll-like receptor 4 (TLR4) is important in cardiac ischemia/reperfusion damage. Moreover, TLR4-knockout mice (TLR4−/−) develop reduced atherosclerosis and cardiac hypertrophy. We investigated the susceptibility of wild-type mice (WT) and TLR4−/− to chronic NO synthase inhibition. Methods: Adult WT (C57BL/6, n = 10) and TLR4−/− (n = 10) were used. The nitric oxide (NO) synthase inhibitor N^̃^−nitro-L-arginine-methyl ester hydrochloride (L-NAME) was added to the drinking water (50 mg/kg/d, 14 days). Mean blood pressure (MBP) was measured by radiotelemetry. Noradrenaline (NE) and calcium (Ca2+) dependent contractility of mesenteric arteries was investigated under isometric conditions. Moreover, heart weight/body weight ratio (HW/BW) and cardiomyocyte size were assessed. Results: The basal MBP was 99.1 ± 6.9mmHg in TLR4−/− and 101.1 ± 2.9mmHg in WT (p<0.05). L-NAME application significantly increased MBP in WT (112.0 ± 1.9mmHg) but not in TLR4−/− (102.0 ± 3.7mmHg). In TLR4−/− HW/BW was larger compared to WT (4.1 ± 0.3 g/kg vs. WT: 4.6 ± 0.2 g/kg; p<0.05) and cardiomyocytes were smaller (TLR4−/−: 3986.6 ± 555.3 units vs. WT: 4692.1 ± 761.1 units; p<0.05). NE- and Ca2+ dependent constriction was significantly reduced in the arteries of TLR4−/− (NEmax, TLR4−/−: 16.1 ± 4.3 mmHg vs. WT: 22.2 ± 4.8 mmHg; Ca2+ max, TLR4−/−: 20.9 ± 4.1 mmHg vs. Ca2+ max WT: 26.1 ± 4.3 mmHg; p<0.05). Conclusion: In L-NAME-induced hypertension, TLR4−/− shows reduced blood pressure, lower HW/BW, smaller cardiomyocytes and decreased vasoconstriction. This suggest that the cardiovascular phenotype of TLR4−/− has characteristics which are of benefit in hypertension research. A constitutive down-regulation of the innate immune system may be speculated. Moreover, our results strengthen the hypothesis that TLR4−/− is characterized by a decreased cardiovascular risk.

2.5 CAPILLARY RAREFACTION PRECEDES THE DEVELOPMENT OF PREECLAMPSIA

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The microcirculation plays an important role in essential hypertension (EH) and preeclampsia (PE). Significant capillary rarefaction (CR) has been reported in individuals with sustained and borderline EH and also in normotensive subjects at high risk of developing EH. It was also shown that women who developed PE had significant CR at the time of pregnancy, 16 weeks before diagnosis. Subjects were studied at 11–16, 20–25, 27–32, 34–38 weeks and 5–15 weeks postpartum. Results: At the end of pregnancy 272 women had normal pregnancy, 16 developed PE (at gestation 35.6 ± 4.8 weeks). The mean difference of – 6 capillaries/field compared to baseline visit, p<0.001. Further rarefaction occurred at 27–32 weeks (mean difference of – 11 capillaries/field, p<0.0001) and 34–38 weeks (mean difference of – 13 capillaries/field, p<0.0001).
2.6 ENDGENOUS HYDROGEN SULFIDE (H2S) AND NOVEL SLOW RELEASING H2S DONOR COMPOUNDS INHIBIT ENDOTHELIAL DYSFUNCTION INDUCED BY TNF-α: OXIDATIVE AND NITROSATIVE STRESS

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Hydrogen sulfide (H2S) is an endogenous endothelium-dependent vasodilator synthesised from L-cysteine by the enzymes cystathionine ß-synthase (CBS) and cystathionine γ-synthase (CSE). Plasma H2S levels decline with age and correlate with higher systemic blood pressure and poorer microcirculatory function in vivo suggesting loss of vascular H2S may mediate the endothelial dysfunction observed during ageing. To test this hypothesis, we have synthesised novel H2S donors such as GYY4137, MC0510 and MC0610 which release H2S in a slow and sustained manner similar to CSE/CBS-derived H2S generation. We exposed human cerebral microvascular endothelial cells to oxidative and nitrosative injury induced by TNF-α, H2O2, oxLDL, the lipid peroxide 4-hydroxynonal (4-HNE) and SIN-1. Endogenous H2S was examined using L-cysteine in the presence and absence of CSE (L-Propargylglycine; PAG) and CBS inhibitors (aminooxyacetic acid; AOA). Pharmacological H2S investigated using H2S donors. Cell death and apoptosis were determined by standard metabolic assays and microscopy. TNF-α (5ng/ml), SIN-1 (2mm), H2O2 (2mm), oxLDL (0.2mg/ml) and 4-HNE (10µM) induced 59.3±6.0%, 73.7±7.1%, 64.5±5.5%, 48.3±4.2% and 46.4±4.1% cell death respectively. With each treatment, cell death was significantly increased when endogenous H2S synthesis was inhibited with PAG or AOA (p<0.001;ANOVA). In sharp contrast, GYY4137, MC0510, MC0610 (0.2mM) or L-cysteine (0.2mg/ml) and 4-HNE (10µM) significantly inhibited cell death (p<0.001;ANOVA). This study strongly suggests endogenous H2S is cytoprotective and its loss may represent a novel mechanism for mediating vascular endothelial dysfunction. Slow releasing H2S donor compounds may offer a viable approach for limiting endothelial damage during ageing and associated vascular pathologies.


3.1 RETINAL PULSE VELOCITY IN MALE SUBJECTS WITH OPTIMAL TO MILD BLOOD PRESSURE VALUES

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Background: Hypertension is associated with an increase in the wall/lumen ratio of resistance arteries. Changes in arterial distensibility and microvascular stiffness contribute to the change in wall/lumen ratio. We hypothesized that microvascular stiffness is associated with hypertension and precedes macrovascular stiffness. We performed a non-invasive measure of retinal pulse wave velocity (rpWV) as measure of microvascular stiffness. Methods: 34 young volunteers with optimal to mildly elevated blood pressure (BP) were examined. Time dependent alterations of vessel diameter were assessed by the Dynamic Vessel Analyzer in a segment of a retinal artery. The data was filtered and evaluated by methods of signal analysis and rpWV were calculated. Office BP, albumin/creatinine ratio and augmentation index (Aix) were assessed. Results: Subjects demonstrated albuminuria below the range of microalbuminuria. rpWV, not Aix, showed a significant association with mean arterial pressure (MAP); p=0.34, p<0.05. Dividing the cohort by the median of rpWV, MAP was elevated in the group with rpWV above the median (rpWV=1.4 mmHg: MAP=97.8±7.4 vs. 103.4±7.2 mmHg, p<0.05). Aix showed no association with MAP or rpWV. Dividing this cohort according to the median of Aix no differences with MAP or rpWV occurred. Conclusion: Retinal PWV as measure of microvascular stiffness differentiates BP in a cohort with optimal to mildly elevated BP. This suggests that blood pressure dependent changes in vascular remodeling may be non-invasively determined by rpWV. This would allow another and potentially earlier marker for microvascular changes beside microalbuminuria. Our results suggest that blood pressure dependent microvascular changes may precede macrocirculatory changes.

Conclusions: Assessment of ventricular rotation by STE is feasible in elderly individuals. Rotation is more marked and more reproducible in the endocardium.
LONG-TERM CARDIOVASCULAR EFFECTS OF ADRENALECTOMY OR MEDICAL TREATMENT FOR PRIMARY ALDOSTERONISM (PA)

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Objective: To investigate the effects of surgical or medical treatment of PA. Methods: After a baseline assessment 136 patients (age 50.7 yrs) with confirmed PA, and 142 optimally treated primary hypertensive patients (PH) as controls, underwent serial echocardiography after adrenalectomy or during medical treatment. Results: PA was due to an aldosterone-producing adenoma (APA) (diagnosed by the “4 corners criteria”), in 65% of the patients and to idiopathic hyper-aldosteronism (IAH) in 35%. At baseline, the surgically- and the medically-treated groups showed a similar high prevalence (52%) of inappropriate LV mass, which fell by 5% at 3.05 yrs follow-up. Both treatments lowered blood pressure (BP) to identical values (136±15/85±8 mmHg); this required more drugs in the medically- than in the surgically-treated PA. The decrease of LV mass index (LVMi) achieved significance in PH and in surgically- but not in medically-treated group. The fall of LVMi involved a decrease of LV end diastolic diameter and volume in both PA groups (p<0.01), and a decrease of LV wall thickness in PH patients, which translated into a decrease of LV stroke work in PH, but not in PA. Conclusions: Treatment of PA reduced BP and induced inward remodeling of the LV, which translated into a prominent decrease of LV work in both adrenalectomized and medically-treated PA patients. The decrease of LVMi did not attain significance in medically-treated PA notwithstanding a higher usage of drugs.

STAGE RENAL FAILURE IN TYPE 1 DIABETIC PATIENTS

MORTALITY, CARDIOVASCULAR EVENTS AND PROGRESSION TO END-STAGE RENAL FAILURE IN TYPE 1 DIABETIC PATIENTS

Mortality, cardiovascular events and progression to ESRD in patients with type 1 diabetes. Materials and Methods: A prospective observational follow-up study including 960 patients with type 1 diabetes (512 men; age-mean±SD: 43.7±11.1; duration of diabetes (mean±SD: 28±11). Of these patients, 458 had diabetic nephropathy (glomerular filtration rate (GFR) 76±34ml/min/1.73 m2) and 442 had persistent normoalbuminuria. Results: During follow-up >173 (20%) patients died of which 109 (12%) were CVD deaths and 75 (8.6%) 16% of patients with diabetic nephropathy developed ESRD. Patients with elevated PP (HR per 10 mmHg increase) had significantly higher all-cause mortality (adjusted HR 1.2 (1.1–1.4); p<0.001), CVD mortality (adjusted HR 1.3 (1.2–1.5); p<0.001), non fatal CVD events (adjusted HR 1.2 (1.0–1.3); p=0.01) and combined fatal and non-fatal CVD (adjusted HR 1.2 (1.1–1.3); p<0.001), (adjusted for sex, age, duration of diabetes, smoking, diastolic blood pressure, Hba1c, cholesterol, UAER, history of CVD and nephropathy status). In patients with diabetic nephropathy elevated PP (HR per 10 mmHg increase) was associated with progression to ESRD (adjusted HR 1.2 (1.0–1.4); p=0.048), (adjusted for sex, age, duration of diabetes, diastolic blood pressure, Hba1c, cholesterol and UAER). Conclusion/Interpretation: Elevated office PP predicts all-cause and CVD mortality, CVD events and progression to ESRD in patients with type 1 diabetes.

MITOCHONDRIAL FUNCTION IN THE FAILING HUMAN HEART

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The cellular pathophysiological mechanism in heart failure (HF) is poorly elucidated. Mitochondrial function may play an important role in the development of the disease. We have characterized mitochondrial function in patients with varying degrees of left ventricular dysfunction (LVD). We hypothesized, that the shift in substrate preference in HF from fatty acid (FA) towards carbohydrate, is caused by decreased capacity for mitochondrial β-oxidation, and that overall oxidative phosphorylation capacity is reduced. Patients were stratified on the basis of left ventricular ejection fraction (LVEF). LVD was defined as an LVEF below 50%. 70 was ruled out by coronary angiography. Myocardial biopsies were obtained from patients undergoing prosthetic valve-, or left ventricular assist device, surgery. Biopsies were excised from the left ventricle after cardioplegia. Mitochondrial respiration was measured in fresh, permeabilized tissue. 9 patients were included in the non-LVD group (LVEF > 58±1%) and 8 in the LVD group (LVEF = 28±8%). Respiratory capacity with FA was decreased in LVD compared with non-LVD (18.1±5.3 vs.5±1.5 pmol-1 sec-1 mg-1, p=0.05). The stimulatory effect of ADP with carbohydrate substrates for complex I was reduced in LVD vs. non-LVD (17.1±2.2 vs. 22±2 pmol-1 sec-1 mg-1, respectively, P=0.05). Maximal respiratory capacity with carboxydrate substrates for complex II and IV was not significantly reduced in LVD vs. non-LVD. LVD was characterized by decreased mitochondrial β-oxidative capacity of medium chain fatty acids and a reduction in complex I capacity. The capacity of complex II and IV was unaffected.

PULS PRESSURE PREDICTS ALL -CAUSE AND CARDIOVASCULAR MORTALITY, CARDIOVASCULAR EVENTS AND PROGRESSION TO END-STAGE RENAL FAILURE IN TYPE 1 DIABETIC PATIENTS

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Background and Aim: Patients with diabetes have an elevated risk of early death due to cardiovascular disease (CVD) and development of end stage renal disease (ESRD). The aim of this analysis is to evaluate whether office pulse pressure (PP) as an estimate of arterial stiffness predicts mortality, cardiovascular events and progression to ESRD in patients with type 1 diabetes.

VITAMIN D LEVELS AND ASYMPTOMATIC CORONARY ARTERY DISEASE IN TYPE 2 DIABETIC PATIENTS WITH ELEVATED URINARY ALBUMIN EXCRETION RATE

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Background: Vitamin D is synthesized in the skin via the conversion of 7-dehydrocholesterol to pre-vitamin D3 under the influence of sunlight. The aim of this study was to determine the prevalence of severe vitamin D deficiency in patients with type 2 diabetes and to examine the association between severe vitamin D deficiency and coronary artery disease (CAD). Methods: A cross-sectional study including 200 type 2 diabetic patients without clinical signs of CAD. Results: The prevalence of severe vitamin D deficiency was 33% (95% CI: 23–43). Conclusions: Severe vitamin D deficiency is prevalent in patients with type 2 diabetes and is associated with an increased risk of CAD. Future studies should investigate whether the use of vitamin D supplementation may reduce the risk of cardiovascular events in type 2 diabetes patients.
severe vitamin D deficiency was 9.5% (19/200). In 70(35%) patients, significant CAD was demonstrated by MPI and/or CAG. Severe vitamin D deficiency was associated with asymptomatic CAD, odds ratio (OR) [95% CI] 2.2 [0.9–5.8]. After adjusting for additional risk factors, OR was 5.0 [1.3–19.5]. The prevalence of CCS>400 was 34% (68/200). Severe vitamin D deficiency was associated with CCS>400, OR 4.3 [1.5–12.1]. The association persisted after adjusting for additional risk factors, OR 4.0 [1.2–13.1].

**Conclusion:** In high risk type 2 diabetic patients with elevated UAER, low levels of vitamin D are strongly and independently associated with asymptomatic CAD.

### 4.3 DIFFERENT VASOCARRIVE EFFECT OF ADHERENT ADIPOSE TISSUE DURING HYPOXIA IN MICE AORTA AND MESENTERIC ARTERIES

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Recent studies propose a paracrine role for perivascular adipose tissue in the regulation of vascular tone. The influence of hypoxia on the effect of brown and white adipose tissue was investigated using isometric tension recording of isolated mice aorta and mesenteric arteries with or without adherent adipose tissue. Hypoxia (bubbling with 95% N2, 5% CO2) relaxed precontracted aorta with brown adipose tissue, while a biphasic response was seen in precontracted mesenteric arteries with white adipose tissue in the presence of indomethacin (10 μM) and nitro-L-arginine (0.1 mM). Only a minimal vasorelaxing effect was observed in both arteries without adipose tissue. Glibenclamide (30 μM) significantly diminished the hypoxic response in aorta, while apamin (1 μM) combined with charybdotoxin (0.1 μM) significantly reduced the hypoxic response in mesenteric arteries. 8-β-sulfophenyl)theophylline (10 μM) did not influence the hypoxic response in both arteries. Removal of the endothelium significantly reduced the hypoxic response in mesenteric arteries, but not in aorta. From these results we conclude that in mice aorta hypoxia induces vasorelaxation in the presence of brown adipose tissue. This relaxation is at least in part mediated by opening KATP channels and independent of the endothelium and adrenergic receptors, suggesting the involvement of the "adipo-derived relaxing factor" (ADRF). In mice mesenteric arteries, hypoxia induces a biphasic response in the presence of white adipose tissue, suggesting the involvement of (a) vasorestrictor(s) and diabrotic(s). The vasodilating response is endothelium-dependent and in part mediated by opening KATP channels, suggesting the involvement of (an) endothelium-dependent relaxing factor(s).

### 4.4 MEAL-RELATED INCREASES IN MICROCIRCULATORY VASOMOTION ARE IMPAIRED IN OBESE INDIVIDUALS

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**Background:** Steady state hyperinsulinemia during a hyperinsulinemic clamp stimulates arteriolar vasomotion and capillary recruitment, which contribute to increased glucose uptake; these phenomena have been shown to be blunted in obesity. This study was designed to investigate whether similar responses can be obtained during physiological hyperinsulinemia.**Methods:** A randomised, placebo-controlled trial was performed in 18 lean and 13 obese subjects, to examine the effects of a glucose drink (75g glucose), a mixed meal drink (60% carbohydrates, 25% proteins, 15% fat) or placebo (tap water) on arteriolar vasomotion in different organs may converge and contribute to CVD. The extracellular matrix protein biglycan (BGN) is involved in cardiovascular disease (CVD) pathophysiology. The aim of the current study was to identify functional analyses of biglycan molecular promoter and transcription factors (TF) involved in its gene regulation. **Material and Methods:** Sequencing of the BGN gene promoter (1199 bp) in 57 CVD patients was performed to characterize its variant structure. Molecular haplotypes (MoHaps) were determined by subcloning. MoHaps and promoter deletion constructs (pGL3-basic) were transfected into endothelial (EA.hy926) and monocytes (THP-1) cells. Cells were kept under basal conditions or stimulated with 10 ng/ml TGF-β1 (24 hours). Gel shift assay (ECL) for polymorphic regions were performed with untreated and TGF-β1-stimulated nuclear extracts. **Results:** We identified three MoHaps: 1 [-578G-151G] and 2 [-578H-151G] and 3 [-578H-151G]. Under basal and stimulatory conditions, MoHaps 2 and 3 were significantly less active (p<0.05) than wt in EA.hy926 and THP-1 cells. Simulation of wt deletion constructs with TGF-β1 increased transcriptional activity (TA) up to 3-fold in THP-1 cells. Performing co-transfection experiments, transcription factor SP1 was shown to increase TA of promoter fragments (c = 2-fold) in EA.hy926 cells. Sequence specific binding of SP1 was demonstrated for MoHap MoHap 2 [578G-151G] and 3 [-578H-151G]. To evaluate the functional importance of the putative SP1 binding sites, we performed luciferase reporter gene assays in Cos7 cells. The luciferase activity was highest in Cos7 cells cotransfected with pGL3-Sp1 and the MoHap constructs. **Conclusion:** The current study suggests that MoHaps and TGF-β1 can influence the transcriptional activity of the BGN gene promoter and that TGF-β1 can up-regulate the BGN expression in endothelial and monocyte-like cells.
in THP-1 cells. Conclusion: (1) bFGF promoter activity is enhanced by TGF-β1 and TF SP1 (2) transcriptional activity of bFGF/MiHaps 2 and 3 is significantly reduced (3) ETS-domain TF Pu.1 binds position G-578A in THP-1 cells.

5.2 ENHANCED MYOCARDIAL ANGIOGENESIS BY GENE TRANSFER WITH TRANSPLANTED CELLS

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Purpose: We evaluate the synergism of transient vascular endothelial growth factor (VEGF) and basic fibroblast factor (bFGF) overexpression on angiogenesis and left ventricular (LV) function after bone marrow cell (BM) transplantation, to determine the potential of multimodal cell-based gene therapy for myocardial repair. Methods: Female Lewis rats underwent coronary ligation 3 weeks before transplantation with male donor BMC, BMC transferred with VEGF5 (BMC-VEGF), bFGF (BMC-bFGF), VEGF and bFGF (BMC + VEGF + bFGF), or medium (control) (n = 3 each group at 3 days, 1 week and 2 weeks; n = 6 each group at 4 weeks; n = 75 total). Three days, 1 week, 2 weeks, and 4 weeks after transplantation, transgene expression was quantitated by realtime polymerase chain reaction, angiogenesis by quantitative histology, and LV function by echocardiography. At 4 weeks, regional perfusion was quantitated with microspheres. Results. The VEGF and bFGF were expressed transiently over 4 weeks. At 1 week, VEGF expression was greatest in BMC-VEGF and BMC + VEGF + bFGF heart (p < 0.05), while bFGF expression was greatest in BMC-bFGF and BMC + VEGF + bFGF rats (p < 0.05). Regional perfusion and vascular densities in the scar were lowest in control, intermediate in BMC, BMC-VEGF and BMC-bFGF, and greatest in BMC + VEGF + bFGF (p < 0.05). Four weeks after transplantation, LV ejection fraction was lowest in control, intermediate in BMC, BMC-VEGF and BMC-bFGF, and greatest in BMC + VEGF + bFGF (p < 0.05). Conclusions: The VEGF and bFGF transgenes were expressed transiently and exerted a powerful synergism on the angiogenic effect of cell transplantation but did not normalized perfusion function. The future of cell transplantation may lie in multimodal cell-based gene therapy, in combination with other novel therapy.

5.3 EARLY DYSTROPHIN DISRUPTION IN THE PATHOGENESIS OF EXPERIMENTAL CHRONIC CHAGAS CARDIOMYOPATHY

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Background: The most intriguing aspect of chronic Chagas cardiomyopathy (CCC) is that it takes a long time to develop after the initial infection by the protozoan Trypanosoma cruzi. Chagas disease is characterized by three phases: acute, latent, and chronic, the heart is the most severely and frequently involved organ. Similarly to CCC, cardiac complications due to cardiomyopathy appear later in life in Duchenne muscular dystrophy due to an absence of or diminished dystrophin. In this study we tested the hypothesis that dystrophin expression could be decreased in the beginning of T. cruzi-infected mice preceding the late development of cardiomyopathy. Material/Methods: Male CD1 mice were infected with 5 × 105 trypomastigotes of the Brazil strain of T. cruzi. Mice were killed 30 and 100 days post infection (dpi) and the expression of dystrophin, fibrosis and dystrophin expression were evaluated. Echocardiography, magnetic resonance and positron emission tomography were evaluated from days 15–100 dpi. Results: At 30 dpi there was an intense and diffuse lymphomononuclear myocarditis, disruption of myofibers, and multiple intracellular parasite nests. The inflammation subsided significantly and parasites were not detected at 100 dpi. Dystrophin immunolabeling was focally reduced or completely lost in cardiac myocytes at 30 dpi, this reduction maintained to 100 dpi. Ejection fraction was significantly reduced at 60–100 dpi. The RV was markedly dilated from 30–100 dpi and the LV wall thickness was increased at 100 dpi. Infected mice displayed greater uptake of glucose from days 15–100 dpi. Conclusion: A late cardiomyopathy developed in mice chronically infected with T. cruzi could be associated with dystrophin loss.

5.4 GENOTYPE-PHENOTYPE RELATIONSHIPS IN VASCULAR EHLERS-DANLOS SYNDROME: ANALYSIS OF 104 CASES

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Background: Vascular Ehlers-Danlos syndrome (vEDS) is a rare autosomal dominant disorder caused by mutations in COL3A1 gene, resulting in a defective type III procollagen. No correlation between the nature of mutations and the course of the disease has been described. Methods: We reviewed clinical features of 104 vEDS patients with a COL3A1 mutation, including 76 probands and 28 relatives. Genetic testing was performed by direct sequencing from cDNA obtained from cultured dermal fibroblasts and/or from peripheral genomic DNA. Results: The 1st major complication was vascular (43.5%), digestive (21%) or obstetrical (14%). Median age at the 1st digestive complication (23[19–32] years) was lower than that for the 1st vascular or obstetrical complication (respectively: 50[36–63] years, p = 0.01). Sixty-six mutations were found, defining 3 groups: group 1 with missense mutations affecting a Gly residue in the G-X-Y triplets, group 2 with splicing mutations, nonsense mutations and microarrangements, group 3 with non Gly missense mutations. Whereas there was no difference in clinical features in the two groups (p > 0.05; corrected for multiple testing) with a later onset of major complications (36 years) and a survival free of major complications estimated at 53% at age 40 years, versus 19% in the whole population (p < 0.001). Conclusion: This study performed on a large series of vEDS patients suggests for the first time genotype-phenotype relationships that may affect genetic counseling. This diagnosis should be considered in subjects with unexplained arterial or bowel rupture, even in absence of a classical morphotype.

5.5 LEVELS OF PLATELET-SPECIFIC MICRONARAS ARE ALTERED IN SUBJECTS WITH PREMATURE CORONARY ARTERY DISEASE

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Coronary artery disease (CAD) is a cause of human morbidity, underlining the need for innovative diagnostic strategies. Platelets play a role not only in acute thrombotic disease but also in the process of atherosclerotic plaque formation. This is exemplified by the beneficial use of anti-platelet therapy. In order to shed some light on the role of platelets in CAD, we aim to identify novel biomarkers, of which microRNAs (miRNAs) could be highly useful. miRNAs exhibit striking tissue- and cell-specific expression, making them attractive biomarkers for diagnostic strategies. Surprisingly, miRNAs are not only present in nucleated cells, but also in plasma and platelets, making them easy accessible. We hypothesized that platelet specific miRNA expression patterns differ between patients with premature CAD and controls. We therefore isolated RNA from platelets of 12 male patients with premature CAD and 12 age- and sex-matched healthy controls and performed miRNA expression profiling (illumina beadchips). Microarray profiling identified 21 of the 893 mature human miRNAs to be differentially expressed between patients with premature CAD and controls (p < 0.05; corrected for multiple testing). From these 214 miRNAs, miR-340*, miR-615–5p, miR-545-9.1, mir-451, miR-454* and miR-624*, were identified with an expression level that was at least 1.5-fold higher in patients as compared to controls. These 6 up-regulated miRNAs were validated with qRT-PCR. In conclusion, isolated platelets of subjects with premature CAD have an altered miRNAs expression profile as compared to controls. These miRNAs may be useful novel biomarkers for the diagnosis of CAD.

5.6 UNDER- EXPRESSION OF THE TWIK-RELATED ACID-SENSITIVE K+ CHANNEL 2 (TASK-2) GENE IN ALDOSTERONE-PRODUCING ADENOMA

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Background: Primary aldosteronism is a common cause of arterial hypertension, but its underlying molecular mechanisms are unknown. Genetic manipulation of a potassium channels class, which generates background or “leak” K+ currents, the Twik-related Acid-Sensitive K+ channel 1 (TASK-1) and 2 (TASK-2), generated a phenotype mimicking human PA, suggesting that a blunted activity of them plays a role in PA. The aim of this study was to measure the expression of TASK channels in adenoma producing adenoma (APA) Methods: Whole transcriptome of 24 APA was compared to that of a normal human adrenal cortical tissue pool (n = 16) by oligonucleotides technique. Data were validated by quantitative real time PCR. Immunohistochemistry was used to investigate TASK expression in tissue. Membrane and cytosolic protein fractions were separated by ultracentrifugation and analyzed by immunoblotting. Dimeterization of TASK channels was investigated by confocal microscopy in adrenal carcinoma cells (HAC15 and H295). Results and Conclusions: The most abundant transcript among TASK channels in both the normal 2G and in APA was TASK-1, followed by TASK-2 and TASK-3. Transcriptome analysis, confirmed by quantitative real time PCR, showed that TASK-2 was consistently underexpressed in APAs, while TASK-1 and TASK-3 were heterogeneously expressed. Immunohistochemistry confirmed the expression of the 3 TASK channels in human adrenal cortex. TASK2 expression was found in membrane and cytosolic protein fractions by immunoblotting and confocal microscopy. Given the high heterogeneity of APA, the understanding of TASK-2 expression could help to consider this gene as a key feature of the mechanisms leading to aldosterone over production of these tumours.
DECREASED URINARY ALBUMIN EXCRETION ASSOCIATED WITH THE NORMALIZATION OF THE CIRCADIAN BLOOD PRESSURE PATTERN BY ANGIOTENSIN RECEPTOR BLOCKADE

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Administration of angiotensin-receptor blockers (ARB) at bedtime as opposed to upon awakening increases the sleep-time relative blood pressure (BP) decline and their efficacy in lowering nocturnal BP. We evaluated the administration-time-dependent effects on urinary albumin excretion of ARB-treatment in subjects with essential hypertension. We studied 815 untreated hypertensive subjects (217 men), 48.7±13.5 years of age, randomized to ARB-therapy treatment either on awakening or at bedtime. BP was measured at 20-min intervals from 07:00 to 23:00h and at 30-min intervals at night for 48h before and after 12 weeks of therapy. The subjects collected their urine during the first 24h of each BP monitoring session. The reduction in awake BP was similar for both treatment-time groups. Treatment at bedtime, however, was significantly more effective in reducing asleep BP (17.2 versus 11.2 mmHg after morning treatment; P = 0.001). The sleep-time relative BP decline was increased towards a more dipping pattern only after bedtime dosing (P = 0.001). Albumin excretion was significantly reduced after treatment, and to a significant larger extent after bedtime dosing (P = 0.032). This albumin reduction was highly correlated with the decrease in asleep BP and the increase in sleep-time relative BP decline, independently of treatment-time. Bedtime administration of ARBs provided higher efficacy than morning treatment in reducing the asleep BP mean, improved the sleep-time relative BP decline towards a more dipper profile, and significantly decreased urinary albumin excretion. This study further documents a relation between decrease in asleep BP (a better prognostic marker of cardiovascular mortality than awake BP) and improved renal function.

TREATMENT OF NON-DIPPER ESSENTIAL HYPERTENSION BY BEDTIME ADMINISTRATION OF ANGIOTENSIN RECEPTOR BLOCKERS

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Non-dipping has been related to increased end-organ injury and cardiovascular morbidity and mortality. Accordingly, there is growing interest in how to tailor the treatment of non-dipper hypertensives. We investigated the administration-time-dependent effect on blood pressure (BP) of angiotensin-receptor blocker (ARB) therapy in non-dipper hypertensive subjects. We studied 453 non-dipper subjects with grade 1–2 essential hypertension (217 men), 53.1±14.2 years of age. Subjects were randomly assigned to receive ARB monotherapy (valsartan, 160 mg/day; olmesartan, 40 mg/day; or telmisartan, 80 mg/day) either upon awakening or at bedtime. BP was measured at 20-min intervals from 07:00 to 23:00h and at 30-min intervals at night for 48h before and after 12 weeks of treatment. The reduction in awake BP was similar for both treatment-times (11.0/8.0 mmHg versus 11.3/9.2 mmHg after morning and bedtime dosing, respectively; P = 0.029). Treatment at bedtime, however, was significantly more effective in reducing asleep BP (16.5/11.9 mmHg reduction compared to 12.8/8.8 mmHg after morning treatment; P = 0.001). The sleep-time relative BP decline was increased by 6.9% (P = 0.001) towards a more dipping pattern only after bedtime dosing (P = 0.032). This albumin reduction was highly correlated with the decrease in asleep BP and the increase in sleep-time relative BP decline, independently of treatment-time. Bedtime administration of ARBs provided higher efficacy in reducing the asleep BP mean, improved the sleep-time relative BP decline towards a more dipper profile, and significantly decreased urinary albumin excretion. This study further documents a relation between decrease in asleep BP (a better prognostic marker of cardiovascular mortality than awake BP) and improved renal function.

ANGIOTENSIN II TYPE 1 RECEPTOR SIGNALING REGULATES MICRORNA EXPRESSION BY Gaq AND ERK1/2 DEPENDENT MECHANISMS

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The Angiotensin II type 1 receptor (AT1R) is a key regulator of blood pressure, body fluid homeostasis and cardiac contractility and is profoundly involved in development of cardiac disease. AT1R signaling involves both activation of heterotrimeric G proteins and signal cascades dependent on β-arrestins. Hormone signaling has been suggested to involve regulation of microRNAs (miRNAs), which are small (~22nt) non-coding RNAs that negatively regulate the stability and translation of mRNA targets. Since several miRNAs have been implicated in cardiac biology and disease, we asked whether miRNAs might be regulated by AT1R activation. Hence, we performed an analysis of AT1R-mediated miRNA regulation and focused on selecting the role of G protein-dependent and -independent pathways. A global miRNA array analysis revealed five miRNAs (miR-29b, –129-3p, –132, –132* and –212) that were upregulated by Angiotensin II (Ang II) treatment in HEK293 cells overexpressing the AT1R. Importantly, three of these miRNAs were also upregulated after AT1R activation of an in vivo cellular culture of human blood vessels with endogenous receptor expression. In contrast, the biased Ang II analogue, [Sar1, Ile4, Ile8] Ang II (SII Ang II), which selectively activates G protein-independent signaling, failed to upregulate these five miRNAs. Furthermore, induction of the Ang Iregulated miRNAs was blocked following Gq/11 and Mek1 inhibition with specific pharmacological blockers. In conclusion, AT1R signaling regulates miRNA expression through mechanisms involving Gq/11 protein and Erk1/2 activation. We thus set the postulate that such miRNAs could be involved in Ang II-mediated cardiac biology and disease.

LACK OF MID-TRIMESTER BLOOD PRESSURE DROP IN NORMAL PREGNANCY

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It is generally accepted that in healthy pregnant women, systemic blood pressure (BP) falls gradually at early gestation, reaching a trough at 22–24 weeks, and rising again reaching pre-pregnancy levels by 36 weeks gestation. In a longitudinal study of microcirculatory changes in pregnancy, we noted an absence of this mid-trimester fall. We therefore prospectively studied this phenomenon in all our subsequent recruits. Methods: We studied 255 women who were normotensive at booking and after delivery. BP was carefully measured under standardised conditions using the semi-automated device Omron® HEM907. Subjects were studied at 12–16, 20–25, 27–32, and 34–38 weeks. Three sitting and 2 standing BP measurements were obtained at 2-minute intervals using appropriate cuff size. We also extracted BP measurements done by midwives from the case notes of 51 women within this cohort and analysed the data to validate our results. Results: Systolic BP progressively increased from the first trimester through to 38 weeks gestation. The increase from baseline at 11–12 weeks was significant when compared to measurements at 22 weeks (mean difference (MD): 2.8 mmHg), 28 weeks (MD: 5.0) and 36 weeks (MD: 7.7 mmHg). Systolic BP progressively increased at 22 weeks (MD: –0.12 mmHg) but increased at 28 weeks (MD: 2.0 mmHg) and 36 weeks (MD: 6.0). In the validation cohort, the systolic BP (p = 0.0001) and diastolic BP showed an increasing trend (p = 0.0001). Conclusions: BP measured under controlled conditions showed a progressive rise in pregnancy, with no significant mid trimester drop. The findings were replicated in measurements in the routine antenatal clinic.
CUTANEOUS ANTI-ISCHEMIC EFFECTS OF 17β-ESTRADIOL AND TESTOSTERONE: STRONG PREVENTION OF SKIN FLAP NECROSIS

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β-estradiol (E2) was found to be protective in many experimental models of myocardial and brain ischemia. More recently, we demonstrated the protective effect of E2 in a mouse model of skin ischemia, mimicking the surgery of skin flaps1. Whereas necrosis appeared in the half portion of the skin flap within one week after surgery in ovariectomized mice, it was reduced up to 10-fold when mice were pre-treated with E2 (treated at least 3 days before the surgery). The beneficial effect of E2 appeared to involve: 1) an increase in skin survival, revealed by measuring viability of *ex vivo* explants and enhancement of the anti-apoptotic Bcl-2 protein expression *in vivo*. 2) a protection of the vascular network, facilitating reperfusion which was found to be accelerated in ovariectomized E2-treated mice, while diffuse hemorrhages characterized untreated mice skin flaps. This protective effect of E2 was mimicked by a treatment with tamoxifen, a selective estrogen receptor modulator (SERM). Male mice were also found to be protected by E2. However, testosterone, as well as dihydrotestosterone, a non aromatizable androgen, reduced skin flap necrosis, strongly suggesting a direct activation of the androgen receptor. Thus, redundancy of action of estrogens and androgens could concour a prevention of cutaneous ischemia, and the precise mechanisms of this protection in males will be presented.

ECR Poster Presentations

PA.01
ALTERATION OF MICROVASCULAR FLOW-MEDIATED DILATATION IN SYRIAN HAMSTERS LACKING δ-SARCOCYLAN IS CAUSED BY ENHANCED OXIDATIVE STRESS

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δ-sarcocyanin mutation induces dilated cardiomyopathy associated with abnormal aortic vascular smooth muscle cell proliferation and apoptosis. We hypothesized that vascular tone might be affected in resistance arteries, which have a key role in the local blood flow. We investigated vascular tone in mesenteric resistance arteries (MRA) isolated from δ-sarcocyanin-deficient hamsters (CHF-147). Vascular structure was not significantly modified in CHF-147. Compared to control P2Y1 receptors Ectodominium-independent and –dependent dilation were not modified in CHF-147. Contractility to KCl was not modified and that to phenylephrine was slightly increased. Basal NO and eNOS expression were not modified in CHF-147. Thus, vascular mechanism to flow was selectively reduced in MRA from CHF-147. Reactive oxygen species levels were higher in CHF-147; reducing this level by using tempol increased FMD in CHF-147 to the level found in control hamsters. COX-2 was increased in CHF-147 and COX-2 inhibition improved FMD. This study suggests that the sarcocyanin complex is selectively involved in flow-mediated dilation thus highlighting its role in endothelial responsiveness to shear stress. Furthermore, the involvement of oxidative stress and inflammation in this defect opens potential therapeutic perspectives to improve endothelial function in myopathies.

PA.02
ROLE OF NUCLEOTIDES, ECTONUCLEOTIDASES AND P2 RECEPTORS IN VASCULAR CONTRACTION AND MYOGENIC TONE

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Background: Extracellular nucleotides promote vascular relaxation and constriction through cell membrane ligand-gated P2X and G protein-coupled P2Y receptors. P2X are activated by ATP while P2Y are differentially activated by ATP, ADP, UTP, UDP or UTP-glucose. We evaluated the role of P2 receptors in vascular responses and investigated their contribution to myogenic tone.

Methods: We used in situ perfusion method to study the role of P2 receptors in vascular responses. We observed that P2 receptors play a role in the control of myogenic tone.

Results: Using in situ perfusion method and HPLC we found that NTPDase1 deletion (EntpD/-/-mice) results in a virtual absence of nucleotides activity in medial smooth muscle. This reduced activity unmasked a potent constrictor effect of UDP and UTP in myogenic tone that seems to be unrelated to NO production. NTPDase1 deletion (EntpD/-/-mice) decreased relaxation to ATP and abolished constriction to ADP. Therefore, NTPDase1 deletion results in a virtual absence of nucleotides activity in medial smooth muscle. This reduced activity unmasked a potent constrictor effect of UDP and UTP in myogenic tone that seems to be unrelated to NO production.

Conclusion: Our data show that NTPDase1 regulates nucleotide dependent vasconstriction and suggest that purinergic signalling may participate in resistance artery auto-regulation. The enhanced myogenic tone observed in EntpD/-/- arteries could be due to facilitated P2Y receptor activation which seems to fully support vascular contraction in response to uracil nucleotides.

PA.03
WHAT IS THE OPTIMAL ANESTHETIC PROTOCOL FOR MEASUREMENTS OF CEREBRAL AUTOUREGULATION IN SPONTANEOUSLY BREATING MICE?

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Autoregulation, an important feature of the cerebral circulation, is affected in many diseases. Since genetically modified mice are a fundamental tool in biomedical research, including neuro(bio)logy also in this species measurements of cerebral autoregulation (CA) are mandatory. However, this requires anesthesia that unfortunately significantly impacts the anesthetic of choice for CA measurements in spontaneously breathing mice.

PA.04
MIDDLE CEREBRAL ARTERYALTERATIONS IN A RAT CHRONIC HYPOPERFUSION MODEL

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Chronic cerebral hypoperfusion (CHP), defined as moderate ischemia, induces microvascular changes that could contribute to the progression of vascular cognitive impairment and dementia in ageing brain. After CHP, as induced with rat bilateral common carotid artery occlusion (BCCAO) in rats, remodelling of the basilar artery has already been reported. We aimed to analyse the effect of CHP on myogenic, mechanical and structural properties of rat middle cerebral artery (MCA). BCCAO was induced in adult–male Wistar rats. After 15 days, animals were anaesthetized and MCA was dissected. Sham-operated animals underwent the same surgical procedure without carotid ligation. MCA structure, mechanical and myogenic properties were assessed by pressure myography, collagen/VI protein expression by immunofluorescence and superoxide anion (O2−) production by ethidium fluorescence. After 15 days of BCCAO the structural parameters wall thickness (p<0.001), wall/lumen ratio (p<0.001) and cross-sectional area were diminished (p<0.01) when compared to sham-operated animals. Nevertheless, mechanical parameters were differently affected by BCCAO: wall stress (p=0.01) was increased whereas stiffness was not modified. The myogenic tone was diminished (p<0.01) and the lumen diameter in active conditions increased (p=0.05) after BCCAO. Production of O2− was similar in both groups of rats and the expression of collagen VI was diminished (p=0.001) in rats submitted to BCCAO. These data suggest that BCCAO induce: i) hypotrophic remodelling of the MCA accompanied by diminished collagen/VI and ii) a decrease in the myogenic tone that seems to be unrelated to O2− as suggested in the same artery after cerebral ischemia/reperfusion.

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PA.05
IN VIVO STUDIES ELUCIDATING THE FUNCTIONAL ROLE OF SOLUBLE GUANYLYL CYCLASE (sGC) AND ITS DIFFERENT ISOFORMS IN VASODILATATION AND PENILE ERECTION

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The nitric oxide/cyclic guanosine phosphate (NO/cGMP) pathway plays a pivotal role in vasodilatation and as such also in penile erection. Recently sGc-activating agents have been put forward as novel therapeutic approaches for hypertension and erectile dysfunction. The existence of 2 physiologically active sGc isoforms (sGcα1 and sGcα2) offers a potentially more selective approach. However more knowledge is required on the functional importance of the different isoforms in vasodilatation. To investigate this we performed in vivo studies using 2 types of transgenic mice, sGcα1ki/ki mice and completely abolished in sGcα1ki/ki mice. While intravenous administration of L-NAME induced an increase in MAP in wild-type mice. These responses were significantly reduced in sGcα1ki/ki mice and completely abolished in sGcα1ki/ki mice. While intravenous administration of L-NAME induced an increase in MAP in wild-type mice, this increase was significantly reduced in sGcα1ki/ki mice and abolished in sGcα1ki/ki mice. Stimulation of cavernosal nerves resulted in frequency-dependent increases in ICP in control mice which were strongly reduced in sGcα1ki/ki mice and abolished in sGcα1ki/ki mice. Equal responses to sGc-independent agents in transgenic mice and their wild-type controls confirmed the specificity of the impaired sGc-related responses. These studies illustrate that NO-induced vasodilatation and penile erection is completely sGc-dependent. While intravenous administration of L-NAME induced an increase in MAP in wild-type mice, this increase was significantly reduced in sGcα1ki/ki mice and completely abolished in sGcα1ki/ki mice. While intravenous administration of L-NAME induced an increase in MAP in wild-type mice, this increase was significantly reduced in sGcα1ki/ki mice and completely abolished in sGcα1ki/ki mice.

PA.06
G-PROTEIN B1 SUBUNITS MEDIATE CALCITONIN GENE-RELATED PEPTIDE-INDUCED EFFECTS

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Endothelin-1 (ET-1) causes long-lasting effects by quasi-irreversible binding to ET1 receptors. We have shown that calcitonin gene-related peptide (CGRP) receptor activation applied in low doses combined with local anesthetics had the best reproducibility. Although with this anesthesia the lower CA limit was lower than with Ketamine/Xylazine and Chloralose as reported in the handout of papers so far dealing with CA in mice we suggest Ethomidate as the anesthetic of choice for CA measurements in spontaneously breathing mice.
promotes dissociation of ET-1 from ET receptors (“terminator effect”) (Meens et al. 2010, in press). This was not mimicked by activators of adenyl cyclase (AC). Here we evaluated whether G-protein coupled subunits (Gq/11) are present in cerebral arterial beds. The anticoagulant heparin (10 µM), forskolin (10 µM) and Gq/11-mediated effects, including inhibition of cyclic AMP accumulation, were observed in cerebral arteries. This suggests that Gq/11 activation is present in cerebral arteries and may act as a negative feedback regulator of ET-1 release.

PA.07

UPREGULATION OF CONTRACTILE ENDOTHELIN TYPE B2 RECEPTORS BY LIPID-SOLUBLE CIGARETTE SMOKING PARTICLES IN RAT CEREBRAL ARTERIES VIA ACTIVATION OF MAPK

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Cigarette smoke exposure is a strong risk factor for cerebral vascular disease. However, the underlying molecular mechanisms to explain this are poorly understood. The present study was designed to examine the hypothesis that endothelin type B2 receptors in cerebral vascular smooth muscle cells might be the molecular site that mediates the cigarette smoke-associated increase in stroke by the involvement of MAPK. Rat cerebral arteries were incubated for 24 h in the presence of lipid-soluble cigarette smoke particles with or without specific inhibitors: MEX specific U0126, p38 specific SB202190, JNK specific SP600125, NF-κB specific BMS- or IMD-0354, transcription specific Actinomycin D, or translation specific Cycloheximide. The specific ET B receptor agonist Sarafotoxin 6c were investigated by a sensitive myograph method. Results were verified by measurement of mRNA with quantitative real time PCR and protein by immunohistochemistry. Organ culture induced transcriptional upregulation of endothelin ETB receptor. This upregulation was further increased at the transcriptional level by addition of lipid-soluble cigarette smoke particles. The organ culture mediated upregulation of endothelin ETB receptors was inhibited by U0126, Actinomycin D, and Cycloheximide. The enhanced upregulation of ETB receptors by lipid-soluble cigarette smoke particles was inhibited by U0126, SP600125, Actinomycin D, and Cycloheximide. The underlying molecular mechanisms involved in this includes activation of MAPK MEK and JNK and transcriptional and translational mechanisms. This may advance us understanding how cigarette smoking induces the risk for cerebral vascular disease.

PA.08

LOCAL ENHANCED CONTRACTILE RESPONSE OF ETB, 5-HT1B AND AT1 RECEPTORS FOLLOWING DISTAL FOCAL PERMANENT OCCLUSION

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A time-dependent up-regulation of the contractile endothelin-B (ETB), 5-hydroxytryptamin-1B (5-HT1B) and angiotensin-1 (AT1) receptors has previously been demonstrated in cerebral arteries following subarachnoid haemorrhage and transient middle cerebral artery (MCA) occlusion. Here we investigate whether a small vascular insult leads to a locally altered receptor expression profile of segments located upstream- and downstream to an occlusion. Focal permanent occlusion by distal MCA ligation was performed on male Wistar rats, wherein the right MCA was accessed by craniotomy and occlusion is verified by laser-Doppler. Controls consist of un-ligated left MCA and sham operated rats. After 24 and 48 hours, MCA segments were investigated for expression changes of ETB, 5-HT1B and AT1 receptors at functional (myograph) level. Ischemic damage was examined by TTC staining. After 24 hours the contracture responses to S6c (selective ETB agonist) and 5-CT (5-HT1B agonist) were significantly stronger in the downstream group compared to the upstream group (13.3 ± 7.4 vs. 3.2 ± 2.1, p = 0.01). The use of the non-specific 5HT1B agonist 5-carboxamidotryptamine (5-CT) is not significantly different in the two groups. Interestingly, most of the changes were observed downstream of the occlusion.

PA.09

RAPID TRANSCRIPTIONAL UPREGULATION OF ETB RECEPTORS IN RAT CORONARY ARTERIES DURING MYOGRAPH STUDIES

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In cardiovascular disease, vasococontractile endothelin ETB receptors are expressed in the smooth muscle layer of coronary arteries. Interestingly, a similar receptor upregulation has been observed after organ culture of arteries. This study aims to examine the time course of the early ETB receptor functional changes in two different coronary artery segments: the left anterior descending (LAD) and the septal coronary arteries (SCA). The function of the ETB receptors was measured during incubation in a wire-myography. The specific ETB receptor agonist Sarafotoxin 6c (S6c) was added to the segment in four (1/10, 1/4, 1/7, and 24 hrs) cumulative concentration curves (1pM to 30 nM). After 1/3 hours incubation the ETB receptor mediated vasconstriction was almost negligible (Emax in LAD 9.5 ± 3.8; SCA 6.3 ± 4.4). However the response developed rapidly. In both vessels 2 and 7 fold increases in the Emax were detected after 4 and 7 hrs of incubation, respectively. The response reached a maximum in LAD after 7 hrs, whereas the response continued to develop in the septal coronary arteries until 24 hrs of incubation. The transcriptional inhibitor, Actinomycin D (4 µM), or the MEK1/2 inhibitor, U0126 (10µM) attenuated the S6c mediated response significantly. The LAD and SCA displayed the ability to rapidly develop an ETB receptor mediated contractile response during incubation in a wire-myography. This study suggests that the functional development of the ETB receptor mediated response depends on a transcriptional upregulation of the receptor and involves the MEK/ERK type of MAPK.

PA.10

EXPRESSONAL CHANGES IN CEREBROVASCULAR RECEPTORS AFTER GLOBAL CEREBRAL ISCHEMIA

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Global cerebral ischemia occurs when the blood supply to the entire brain is diminished and may result in stroke and permanent brain damage. It occurs commonly during cardiac arrest, which in Europe affects 1000 persons daily. We have previously demonstrated upregulation of vasococontractile receptors in arteries supplying ischemic tissue upon focal cerebral and myocardial ischemia, a phenomenon that contributes to reduced perfusion and worsened ischemic damage. On this basis, we aimed to investigate whether such receptor upregulation also occurs in cerebral arteries after global cerebral ischemia. Global cerebral ischemia most severely affecting the forebrain was induced in rats by 15 minutes of two-vessel carotid artery occlusion combined with systemic hypotension. 48 hours later, cerebral arteries were isolated and contractile responses to endothelin-1 (ET-1) and 5-carboxamidotryptamine (5-CT, a 5-hydroxytryptamin (5-HT) analogue) were assessed by wire myography. Neurological deficits were evaluated daily by grip strength and a rotating pole test, showing significant deficits in ischemic rats. Contractile responses to ET-1 and 5-CT in middle cerebral arteries (MCA) and anterior cerebral arteries (ACA) were enhanced in ischemic rats compared to sham-operated rats resulting in leftwards shifted dose-response curves. In ACA, the maximal responses to ET-1 and 5-CT were increased to 137±17% and 204±33%, respectively, of sham-operated rats. In contrast, contractile responses in BA were unchanged by the ischemia. In conclusion, the ischemia-induced enhanced contractility observed in MCA and ACA but not BA suggest that contractile ET-1 and 5-HT receptors are upregulated selectively in the arteries supplying the forebrain, which is most severely ischemic.

PA.11

DOXYCYCLINE DOES NOT INTERFERE WITH THE POST-THROMBOTIC NEONITIA DEVELOPED IN AN EXPERIMENTAL MODEL OF INTRINSIC STENOSIS INVOLVING THE SIMULATION OF AN AORTIC ECCENTRIC ATHEROSCLEROTIC PLAQUE

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Smooth muscle cell (SMC) proliferation and migration contribute to neointima formation. This migration has been related to matrix degradation through the production of metalloproteinases (MMPs), specifically MMP-2 and MMP-9. Objective: We described the time sequence of post-thrombotic neointima development surrounding a mushroom hemispherical plug inserted into the aorta and discuss the participation of MMP-2 and MMP-9. Methods: Wistar rats were divided into four groups: sham, sham-operated, treated-operated. The treated groups were administered doxycycline. Euthana-
BY GUEST ON AUGUST 30, 2017

ENDOTHELIAL DISFUNCTION IN ACE2-GENE-DELETED MICE - ROLE OF OXIDATIVE STRESS AND NITRIC OXIDE IMBALANCE

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Introduction: Accumulating evidence indicates that ACE2 plays a critical role in cardiovascular homeostasis, and its altered expression is associated with major cardiac and vascular pathophysiologies. Recent studies suggest that oxidative stress, which is elevated in cardiovascular disease, contributes to endothelial dysfunction. The aim of the present study was to evaluate the endothelial function and assess the vascular redox balance in ACE2 gene-deleted animals. Methods and Results: Male adult C57Bl/6 and ACE2 gene-deleted mice (ACE2−/−; 20 to 24 weeks old) were used in this study. To analyze the endothelial function in vivo, endothelium-dependent and independent agents (acetylcholine and sodium nitroprusside, respectively) were applied in vivo to the descending thoracic aorta and blood pressure was monitored. Endothelial function turned out to be significantly impaired in ACE2−/− mice compared to C57Bl/6 animals. Western blot analysis of aorta revealed decreased levels of phosphorylated eNOS (Ser1177) in ACE2−/− mice in comparison to controls. In addition, the aorta NO levels, assessed by DAF-2, were reduced in ACE2−/− animals. Moreover, these mice presented a lower plasma and urine nitrite concentration in comparison with controls, as detected by a fluorometric assay. However, the arginine activity in this tissue was similar in both groups. Consistent with this, lipid peroxidation was significantly increased and SOD activity was decreased in the aorta homogenate of ACE2−/− mice compared with controls, indicating impaired antioxidant capacity in these animals. Conclusion: All together, these data demonstrate that oxidative stress and NO imbalance are involved in the mechanisms of endothelial dysfunction in ACE2−/− mice.

ENDOTHELIN RECEPTOR ACTIVATION REVERSES HYPOXIC VASODILATION IN PORCINE LARGE CORONARY ARTERIES

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The study investigated the role of endothelin-1 (ET-1), the nitric oxide (NO) pathway, and radical oxygen species in hypoxia-induced vasodilation of large coronary arteries. Porcine coronary artery segments were mounted for functional studies, tissue concentrations of ET-1 and asymmetric dimethylargine (ADMA) were measured, and NO concentration was measured with a microsensor. In prostaglandin F2α-contracted segments with endothelium, gradual lowering of oxygen tension from 5% to 0% resulted in vasodilation. The response to 0% lowering was rightward shifted in segments without endothelium, but not at 0% O2. The endothelin receptor antagonist SB272124 (10 μM) increased hypoxic dilation and exogenous ET-1 reversed hypoxic vasodilation in segments with and without endothelium. The free tissue ET-1 concentration in the arterial wall was unchanged 1% O2 versus 21% O2. Without affecting basal NO, hypoxia increased NO concentration in PGF2α-contracted arteries, and a NO synthase inhibitor, nitro-L-arginine (300 μM) reduced hypoxic vasodilation. ADMA concentrations were unchanged by hypoxia. The superoxide scavenger tiron (10 μM) and the putative NADPH oxidase inhibitor apocynin (10 μM) reversed the hypoxic vasodilation and reduced NO synthase activity, but not in segments without endothelium. The present study suggests that ET-1 plays an important role in hypoxic response, since endothelin receptor activation reverses while endothelin receptor antagonism markedly enhances the endothelium-independent hypoxic vasodilation. Endothelium-derived NO may counteract the effects of endothelin-1 and radical oxygen species during hypoxia.

CYCLOPHILIN A AND ENDOTHELIAL DYSFUNCTION IN HYPOXIC PULMONARY HYPERTENSION

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Background: Cyclophilin A (CyPA) contributes to vascular remodeling in the systemic circulation in animal studies (Satoh et al., 2008). CyPA is also upregulated intracellularly in endothelial cells (ECs) exposed to hypoxia (Ostergaard et al., 2009) and secreted in response to reactive oxygen species. Chronic hypoxia in the lung leads to pulmonary hypertension associated with endothelial dysfunction and vascular remodeling. Hypothesis: CyPA is upregulated and involved in the development of EC dysfunction in the pulmonary circulation of chronic hypoxic rats. Methods: Western blots for CyPA were performed in lung tissue and pulmonary arteries from hypoxic and normoxic rats. The effect of exogenously added CyPA in physiologically relevant concentrations (10−3 to 300 nM) on rat pulmonary small arteries (300–500 μm) was investigated by use of micromanipulation. Concentration-response curves were constructed for acetylsalicylic acid, a nitric oxide donor (SNP), and an opener of IK Ca- and SK Ca2+-channels in pulmonary arteries from both normoxic and chronic hypoxic rats. Results: Western blotting showed the presence of CyPA in the lung from both normoxic and chronic hypoxic rats. However, further addition 100 nM of the BK Ca-channel blocker iberiotoxin completely inhibited the effect on the endothelium-dependent vasodilation in rat pulmonary small arteries.

INVolVEMENT OF HYDROGEN SULFIDE IN ENDOTHELIN-DEPENDENT VASODILATION

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Background: Hydrogen sulfide (H2S) is an endogenously produced gas with many physiological functions including involvement in the regulation of blood pressure. Objective: The present study investigated the mechanisms underlying H2S-induced vasodilation, and addressed whether it is involved in endothelium-dependent vasodilatation in rat small mesenteric and pulmonary arteries. Methods: Mesenteric and pulmonary arteries from rats were mounted in micromanipulation. Measurements and relaxations were monitored using the pressure myography. Concentration-response curves were constructed for acetylcholine, a nitric oxide (NO) donor (SNP), and a opener of IK Ca and SK Ca2+-channels (NS309) in pulmonary arteries. Results: Application of NaHS relaxed mesenteric arteries with an EC50 of 3.85×10−6 M. Removing endothelium, inhibition of KCa1.5 channels and NO synthase did not inhibit NaHS relaxation in mesenteric or pulmonary arteries. Simultaneous measurements of calcium and relaxation revealed that NaHS induces relaxation without changes in smooth muscle calcium. In mesenteric arteries acetylcholine relaxation was reduced in the presence of PGP and AOA both in the absence and the presence of indomethacin and nitro-L-arginine. In pulmonary arteries, the combination of indomethacin and nitro-L-arginine inhibited acetylcholine relaxation, and there was no further effect by adding PGP and AOA. Conclusions: H2S induces vasorelaxation in both rat mesenteric and pulmonary arteries, but is only involved in acetylcholine vasodilatation in rat mesenteric arteries. H2S-induced relaxation was endothelium-independent and may involve desensitization of the contractile apparatus in the vascular smooth muscle.

THE BKCA-CHANNEL IS PART OF THE EDHF-RESPONSE IN HUMAN SUBCUTANEOUS RESISTANCE ARTERIES

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Background: In human resistance arteries the mechanism responsible for endothelium-derived hyperpolarization (EDHF)-response has yet to be fully elucidated. Earlier work based on the use of charybdotoxin (ChTx) and apamin has indicated important roles for the calcium-activated potassium channels SKCa and IKCa. However ChTx blocks BKCa in addition to IKCa, and the role of the BKCa-channel is therefore unclear. Methods: Human subcutaneous abdominal fat biopsies were obtained from healthy kidney donors. Resistance arteries were mounted on a wire myograph. Their response to increasing concentrations of acetylcholine (ACH) were obtained following noradrenaline (NA) precontraction. Results: Our data reveals that blocking the cyclooxygenase (COX) with 3 μM indomethacin and eNOSs with 100 μM L-NAME significantly shifted the sigmoid curve to the right (-logEC50 7.00 vs. 5.97 ± 0.17; p < 0.001). We further blocked the SKCa and IKCa-channels with 0.5 μM apamin and 1 μM TRAM64. Surprisingly we found no significant effect of these drugs (-logEC50 5.74 ± 0.02 against 5.44 ± 0.27; p < 0.38). However, further addition 100 nM of the BKCa-channel blockeriberiotoxin completely...
abolished the vasodilatation (contraction at 30 μM ACh 22.5±11.6% vs. 99.6±0.2% – p<0.001). Discussion: Our results show that pharmacological inhibition of the SKCa and IKCa channels is not sufficient to inhibit the EDHF-response. However blockade of the BKCa channel on top of SKCa and IKCa inhibition resulted in complete abolishing of the EDHF which suggests a pivotal role of the BKCa channel in EDHF-mediated relaxation of human subcutaneous resistance vessels.

VASORELAXANT EFFECT INDUCED BY A GRAPE POMACE EXTRACT OBTAINED BY A NOVEL ENZYMIC PROCESS

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Epidemiological and clinical data have extensively demonstrated the beneficial effect of grape extracts and polyphenol-rich foods in the cardiovascular function. Our aim was to evaluate the vasoactive properties of a new grape pomace extract (GPE) obtained by a novel enzymic procedure. GPE was tested in isolated rat aortic rings disposed in organ baths. Addition of GPE (0.1–100 ppm) induced a significant dose-response vasodilatation in phenylephrine-contracted aortas (Emax 5.00 %; EC50 65.50 ± 15.00 ppm). This response was abolished by endothelium removal (Emax = 16.20±4.23 %). Inhibition of nitric oxide (NO) synthesis by Nω-nitro-L-arginine (L-NAME, 300 μM) completely attenuated GPE-induced dilatation. GPE (30 ppm) also decreased phenylephrine-evoked contraction curves (30.68 ± 5.07 % of reduction) and this attenuation was reverted by the presence of L-NAME and in endothelin-1-mediated aortas. Vasocostriction evoked by exposition of the arteries to pro-oxidant agents such as diethyldithiocarbamic acid (1mM) and endothelin-1 (10mM) was notably attenuated by GPE (30 ppm). These preliminary data evidence that this newly obtained GPE induces a potent endothelium- and NO-dependent vasodilatation in isolated rat aorta. Moreover, GPE seems to restore the impairment of endothelial function under conditions of high oxidative stress. The rich composition of GPE in antioxidants molecules and the vascular effects shown in this study, suggests the potential use of the newly obtained GPE as a co-adjuvant in therapies against cardiovascular diseases.

VASCULAR CHANGES ON MESENTERIC ARTERIES FROM SENESCENCE-ACCELERATED MICE

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Age is recognized as an important risk factor for cardiovascular diseases and alterations in vascular properties are involved in their initiation and progression. Senescence accelerated prone (SAM-P) and resistant (SAM-R) mice have proven valuable in elucidating vascular changes during aging. First order mesenteric arteries from 7 month-old SAM-P and SAM-R female mice were used. Reactivity experiments were performed in a wire myograph and myogenic response at 60 – 80 mmHg was decreased (p<0.05) in SAM-P8 than in SAM-R1. Conversely, NAD(P)/H-oxidase activity was enhanced (p<0.05) in SAM-P8. No differences between groups were observed on the relaxing curves to acetylcholine (ACh, 1nM-10μM), but the contraction to KCl (100mM) was greater (p<0.01) in SAM-P8 (8.0±4.5 mM, n=17) than in SAM-R1 (2.5±0.5, n=11) mice. Phenylephrine induced vasoconstriction in the presence of the NO synthase inhibitor L-NAME (100μM) was greater in SAM-R1 than in SAM-P8 (p<0.01), suggesting a greater bioavailability of NO in aorta from SAM-R1. The diminished NO bioavailability in SAM-P8 is likely due to increased NAD(P)/H-oxidase-derived production of nitric superoxide. However, the lack of difference on ACh vasodilatation seems to indicate that endothelial mediators other than NO may be participating on ACh response and could be modified by age.

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EFFECT OF EXTRACELLULAR PH ON INTRACELLULAR PH AND NITRIC OXIDE PRODUCTION FROM RAT AORTA ENDOTHELIUM AND VASCULAR SMOOTH MUSCLE

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Background: Changes in pH can disturb endothelial cells (EC) homeostasis. We investigated the effect of extracellular pH changes (pHe) on intracellular pH (pHi) and NO production from rat aorta EC and vascular smooth muscle (VSM). Methods: Medium acidification (pH 7.0 and 6.5) was induced by HCl addition while alkalization (pH 8.0 and 8.5) by NaOH; pH and NO production were evaluated in isolated EC and aorta sections by SNARF-1 (10 μM) and DAF-MA (5 μM). Isolated EC fluorescence was evaluated by flow cytometry while aorta sections were studied by confocal microscopy. Results were expressed by fluorescence intensity difference (ΔF= F-Fo); fluorescence increase indicates NO increase or pHi change in the same pHe direction. Results: Extracellular alkalization promoted EC NO (10.33±0.69) and VSM (6.57±2.27) pHi increase; extracellular acidification promoted VSM pH decrease (42.80±13.65) but not in the EC. When NO production was evaluated in isolated EC, both alkalization (4346.60±596.81) and acidification (2093±272.12) promoted NO increase. In aorta sections, alkalization (pH 8.0) and acidification (pH 7.0) promoted VSM NO increase (15.03±4.83 and 48.77±6.94 respectively) with simultaneous decrease in endothelium (-19.72±6.44 and -35.63±15.34 respectively); pH changes to 6.5 and 8.5, increased endothelium (20.93±10.88 and 61.1±1.57 respectively) and VSM NO (29.49±11.96 and 22.95±5.11 respectively). In endothelium-derended aorta sections, extracellular pH did not affect NO production. Conclusion: Extracellular alkalization promotes endothelium and VSM pH increase while acidification does not. Alkalization induces NO endothelium production that can be mediated by intracellular alkalization, however, NO endothelium production induced by acidification was not affected by pH changes.

HIGH ENDOTHELIAL SHEAR STRESS PRESERVES ENDOTHELIAL CELL AND LEADS TO RUPTURE-PRONE PLAQUES

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Background: Previous work showed that high shear stress localized on the upstream of the maximum stenosis in the carotid artery is associated with clinical instability in patients with coronary artery disease (CAD). However, high shear stress is Endothelial Function Protective. Our hypothesis is that high shear stress could have beneficial effects on endothelial cells during unstable plaque formation. Methods: We used perivascular silastic collars to place around the left carotid arteries of rabbit (n=6) and then the animals received a Western-type diet (1% cholesterol and 5% lard) 8 weeks after surgery. And then shear stress was measured. The ratio of intraplaque hemorrhage or necrosis and the percentage of collagen were calculated. Endothelial cell integrity and function was evaluated. Results: Elastic silastic collars induced regions of high shear stress in carotid arteries on the upstream and low shear stress on the downstream. The plaque on high shear stress showed intraplaque hemorrhage and necrosis (83% versus 0%, p<0.01), contained less collagen (10±0.5% versus 33±1.2%, p<0.01) than did low shear stress lesions. The endothelial cell on the high shear stress is integrity; normal, and high NO expression, while the low shear stress has got destruction. Otherwise, the low shear stress induced the endothelial apoptosis, dysfunction, and even ablate. Conclusion: We believe that this model of collar-induced acceleration of carotid rupture-prone plaques and endothelial protection may serve as an excellent model for atherosclerosis plaque rupture. This study also raises a question as to whether endothelial cell dysfunction is critical for plaque formation.
MESENTERIC ISCHEMIA INDUCES STRUCTURAL AND MECHANICAL ALTERATIONS ON MESENTERIC RESISTANCE ARTERIES

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Background: Ischemia-reperfusion (IR) alters cerebrovascular structural, mechanical and functional properties in rats. Some of these changes have been related with enhanced superoxide anion (O$_2^-$) production, mainly due to reperfusion. Increases on endothelin-1 (ET-1) plasma levels after IR have also been reported. Aim: To analyse the effect of superoxide mesenteric occlusion (90 min) followed by reperfusion (24h) on structural and mechanical properties of mesenteric resistance arteries (MRA) from male Wistar-Kyoto rats. Methods: Vascular structure and mechanics were assessed with pressure myography. The expression of collagen VIII was measured by immunofluorescence. The cellular distribution of the vascular wall and the elastin organization of the internal elastic lamina (IEL) were measured using either antibodies and/or endothelin antagonists are necessary to elucidate the role of ET-1 and oxidative stress on the observed alterations. Supported by DGCYT (SAF2007– 60406); LC and AMM were supported by UAB

PC.02

CHRONOTHERAPY WITH A FIXED VALSARTAN/HYDROCHLOROTHIAZIDE COMBINATION: IMPROVED ASLEEP BLOOD PRESSURE CONTROL WITH BEDTIME DOZING

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Previous studies have documented a significant change in dose-response curve, increased proportion of controlled hypertensive patients, and improved efficacy on nighttime blood pressure (BP) when valsartan was ingested at bedtime, as compared to upon-wwaking. However, no study has yet investigated the chronotherapy effect of a once-daily dosing in the administration-time dependent antihypertensive efficacy of valsartan-hydrochlorothiazide (HCTZ) combination. We studied 204 hypertensive subjects (85 men), 49.7±11.1 years of age, randomly assigned to receive valsartan (160 mg) monotherapy either upon awakening or at bedtime for 12 weeks. Due to uncontrolled ambulatory BP, HCTZ (12.5 mg/day) was added to valsartan as a single-pill formulation, maintaining the original circadian time of treatment, for another 12 weeks. BP was measured every 20-200h from 07:00 to 20:00 and every 30-30 min at night for 48h before treatment and after each period of 12 weeks of therapy. The efficacy of valsartan/HCTZ on the awake BP mean was similar for the two treatment-time groups (P>0.682). There was a greater efficacy with bedtime dosing in regulating asleep systolic BP (P=0.015) as well as asleep pulse pressure (PP; P=0.007 between groups). The sleep-time relative systolic BP decline was significantly increased (P<0.001) and the prevalence of non-dipping reduced from 59 to 23% (P=0.001) only when valsartan/HCTZ was ingested at bedtime. In patients uncontrolled with valsartan monotherapy, ingestion of valsartan/HCTZ combination may be preferred at bedtime due to the associated increase in in-sleep time relative BP decline and the decrease in ambulatory PP, both relevant markers of cardiovascular risk.

PC.03

HEME OXYGENASE-1 INDUCTION IS DIFFERENTIALLY INVOLVED IN FLOW (SHEAR STRESS)-DEPENDENT REMODELLING OF RAT RESISTANCE ARTERIES

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Heme oxygenase-(HO-1) is induced by hemodynamic forces in VSM and endothelial cells. We investigated the mechanisms regulating HO-1 induction in flow (shear stress)-dependent remodelling of rat mesenteric resistance arteries (MRA). 24th centimeter MRA were submitted to high (HF), low (LF) or normal blood flow (NF). 2 or 14 days later, arteries were isolated and mounted in an arteriograph. Results: HO activity inhibitor, Sn-protoporphyrin (SnPP), abolished diameter enlargement in HF arteries. However, arterial narrowing was accentuated in LF arteries by SnPP (diameter decrease: 32.3% ± 3.3% vs n=6, SnPP vs LF y= 22.2±2.3; n=5, in CONT-LF, compared to NF arteries), without effect on media thickness. 2 days after arterial ligation, HO-1 mRNA increased in both HF and LF vessels, in association with a significant reduction in mitochondrial aconitase activity, used as a biomarker for oxidative stress, in HF arteries only (by 21% ± 2.4; n=6, reported to NF arteries). Inhibition of macrophage infiltration (Clodronate), decreased HO-1 induction in LF, but not in HF arteries. Similarly, the inhibition of NADPHox activity (Apocynin) decreased HO-1 induction in LF, but not in HF arteries. Finally, in MRA cunnulated in an arteriotomy, HO-1 mRNA was induced by PSs in a flow-dependent manner, and was abolished by L-NAMe, catalase, or the mitochondrial electron transport chain inhibitors, Antimycin A and Rotenone. Conclusion: HO-1 is involved differentially in HF (positively) and LF remodelling (negatively). In LF arteries, HO-1 induction requires macrophage infiltration and it is mediated by NADPHox-derived species. In HF arteries, HO-1 induction is rather dependent on shear stress-generated NO and mitochondria-derived H$_2$O$_2$.

PC.04

THE IMPACT OF RENAL DAMAGE AND ANTIVIRAL THERAPY ON ARTERIAL STRUCTURE AND FUNCTION

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Purpose: Antiretroviral therapy (ART) has dramatically reduced AIDS-related mortality, but it has been associated to an increased cardiovascular risk, even in absence of hypertension. Aims of this study was to describe the influence of renal damage (RD) and ART on arterial function and structure. Methods: We studied 4 groups of normotensive, nonmocholesterolemic, euglycemic patients; one of HIV+ on ART with RD (A; n=25; age 50.2±10.4 years; means ±SD), one of HIV+ on ART without RD (B; n=25; 49.4±6.2 years), one of HIV- not on ART and without RD (C; n=13; 40.0±6.3 years) and one of healthy controls (D; n=25; 50.0±6.8 years). RD was defined by microalbuminuria and eGFR < 60 ml/min. Arterial stiffness was measured by atero-femoral Pulse Wave Velocity (PWV), central BP by tonometry (Sphygmocor), carotid IMT and distensibility were measured by semi-automatic echotracking. Results: A group showed higher aortic SBP and DBP compared to B group. C group showed lower aortic SBP compared to D group. PWV was similar in A and B groups compared to others. Aortic distensibility was significantly impaired in A compared to B and C groups (no data for D group). IMT didn’t show any difference among groups. Conclusions: HIV+ patients are characterized by arterial functional abnormality that might account for their increased cardiovascular risk due to either ART and/or presence of RD.

PC.05

AT2 RECEPTOR STIMULATION PREVENTS AORTIC THICKENING AND PULSE WAVE VELOCITY INCREASE IN L-NAMED HYPERTENSIVE RATS

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There is an unmet need for novel ways to prevent target organ damage, such as arterial remodelling, in order to achieve further cardiovascular risk reduction in hypertensive patients. Increased pulse wave velocity represents an independent cardiovascular risk factor. We investigated, whether the novel, non-peptide agonist of AT2 receptor, compound 21, was able to prevent arterial remodelling in NO-deficient hypertension induced by L-NAMe administration. 3 groups of male adult rats were investigated (n=10 in each): control, L-NAMe (50 mg/kg/day), L-NAMe + compound 21 (0.3 mg/kg/day). Blood pressure was measured non-invasively by tail-cuff plethysmography each week. After 6-weeks, the animals were anaesthetised and two micro-tip (Sampco Preci) catheters were introduced to the proximal and distal end of the aorta for haemodynamic measurement. Intraperitoneal pulse wave was recorded by both catheters simultaneously and its velocity (PWV) was calculated. Wall thickness and inner diameter were determined by morphometry in hematoxyline-eosine-stained aortic cross-sectional slices. We observed that L-NAMe-induced hypertension was associated with increased PWV, reduced inner diameter and increased wall thickness. Treatment with compound 21 minimally attenuated blood pressure and did not affect the aortic inner diameter. However, it attenuated PWV increase and the aortic thickening. PWV strongly correlated with aortic wall thickness. We conclude that PWV correlated with aortic wall thickness and seemed to be independent from NO production, blood pressure level or vessel diameter. Compound 21 prevented the increase in PWV and might therefore contribute to blood pressure-independent cardiovascular risk reduction. (EC 7th FP PIFE-GA-2009-237834 COME-in-CARE)

PC.06

PROLYLCARBOXYPEPTIDASE DEFICIENT MICE REVEAL ITS MULTIPLE FUNCTIONS

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Prolylcarboxypeptidase (PRCP, angiotensinase C, EC.3.4.16.2) is an enzyme cleaving preferentially peptides with a penultimate proline at the carboxyterminal side. It was shown...
to catalyze the hydrolysis of angiotensin II to angiotensin-1-7, the degradation of the kinin B1 receptor agonist des-Arg9-Bradykinin and possibly activates plasma kallikrein. More recently α-melanocyte-stimulating hormone (αMSH) was added to the list of PRP substrates. To study the in vivo function of this enzyme we generated a PRP knockout mouse (PRCP−/−). PRCP-deficient animals showed normal basal blood pressure values and no change in plasma levels of angiotensin-1-7 or 1-7 levels comparing knockout and wildtype animals. Suppression of the angiotensin-1-7 level in extracts of PRCP−/− mice was reduced than two times increased. Measuring the spontaneous blood pressure response to i.v. angiotensin II injection revealed a genetic background specific phenotype: While PRCP−/− mice on FVB/N background showed no difference to their wildtype controls, knockout mice on C57Bl/6 background increased blood pressure 14 mmHg higher than controls. Moreover PRCP−/− mice revealed a clear body weight phenotype. Here we could show that feeding these mice a high fat diet does not lead to body weight gain in PRCP−/− mainly due to reduced fat deposits as recorded by NMR-based body analysis scan. Detecting PRP expression in brain areas associated to control feeding behaviour and double expression of pomegranate enzyme, encoded αMSH in PRCP−/− mice we propose αMSH as a new substrate of PRCP. Taken together PRCP−/− mice reveal multiple functions of this protein in cardiovascular and metabolic regulation.

**EFFECTS OF PROREIN-DERIVED PEPTIDES ON THE PROLIFERATION OF HUMAN CULTURED SAPHENOUS VEIN SMOOTH MUSCLE CELLS**

Akunuri S, Christofi F, Wijetunge S, Hughes AD

**Background:** Prorenin is the precursor of renin, but also acts via the prorenin receptor. Prorenin peptide (PRP) and its rodent analogue, HRP, are fragments of prorenin that inhibit renin effects in some, but not all studies. We examined the effect of these peptides on proliferation of human saphenous vein smooth muscle cells (HSVSMC). Methods: Peptides and other agents were used for 24h to randomly cycling HSVSMC and cell number was measured using a colorimetric method. Proliferation was calculated as % cell number (cf control), effects of peptide - antagonists were expressed with respect to renin antagonist alone. Data are mean±s.e. mean of (n) observations. Results: PRP induced a concentration-dependent increase in cell number (-logEC50 = 6.6±0.2 M). HRP (10−6 M) also increased cell number. The effect of these peptides was not additive and was comparable to PDGF-BB (10ng/ml) (Table). The effect of PRP was inhibited by PD 98059 (10 M), an inhibitor of MEK, and LY 294002 (10 M), an inhibitor of PI-3-K. Conclusions: Prorenin-derived peptides, PRP and HRP, increase proliferation of HSVSMC. This involves actions on MAP kinase and PI-3-K and suggests that these peptides may have a (partial) agonist action on the prorenin receptor, which could contribute to previous contradictory findings with these peptides.

<table>
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<tr>
<th>PDGF</th>
<th>PRP</th>
<th>HRP</th>
<th>HRP + PRP</th>
<th>PD + PRP</th>
<th>LY + PRP</th>
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<tbody>
<tr>
<td>115±6*</td>
<td>122±3**</td>
<td>124±2**</td>
<td>122±2</td>
<td>100±16</td>
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**CYSTAMINE REDUCES BLOOD PRESSURE IN SHR AND ATTENUATES INWARD EUTROPHIC REMODELLING IN VITRO**

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Tissue transglutaminase (t-TG) is involved in small artery remodelling and the organic disulfide cystamine (CYS) has been demonstrated to inhibit t-TG competitively. We aimed to study if inhibition of t-TG could reduce blood pressure and reverse eutrophic inward remodelling of small arteries in SHR. Methods: Organ culture and wire-myograph setups were used to study in vitro inhibition of t-TG, with CYS, in SHR and WKY small mesenteric arteries. In vivo treatment with CYS (80 mg/kg/day) or amlodipine (10 mg/kg/day) was performed with osmotic pumps in adult SHR. Hemodynamic parameters were determined with telemetry (DSI) and small artery structural and functional characteristics determined. [-cys] was determined with a liquid chromatography setup. Results: Small artery lumen diameter was reduced by 7.2±1.9% in SHR and 3.4±3% in WKY, indicating significant (-p<.05), (-p<.001) inward remodelling following in vitro activation with ET-1. In presence of CYS, activation caused a 1.2±0.9 reduction in SHR passive lumen diameter, compared to 2.5±1.0 % increase in WKY, indicating significant (-p<.05) attenuation of inward remodelling by CYS, with a reduced potency in SHR. In vivo administration of CYS (80 mg/kg/day) to SHR reduced in blood pressure, with no concomitant alterations in small artery structure. [-cys] was 2.47±0.036 nmol/ml and confirmed appropriate presence of CYS. Conclusion: CYS reduces blood pressure in SHR, but does not reverse vascular remodelling after 3 weeks administration. t-TG is involved in inward remodelling of SHR and WKY small arteries and CYS attenuates remodelling in vitro, with a reduced potency in hypertensive rats.

**DISPROPORTIONALLY IMPAIRED MICROVASCULAR STRUCTURE IN MILD HYPERTENSION**

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Essential hypertension (EH) is characterized by increased microvascular resistance. We investigated whether coronary (C-Rmin) and forearm minimum (F-Rmin) vascular resistances are affected proportionally to the blood pressure (BP) level, using echocardiography and plethysmography. 75 never treated EH patients (average age 48 yr, 60% males) without cardiovascular disease and with 24-h systolic BP (SBP) ≥ 130 mmHg or diastolic BP (DBP) ≥ 80 mmHg were assigned into two groups according to their median 24-h SBP (133 mmHg: either Very Mild EH (125/79 mmHg) or Mild-Moderate EH (145/98 mmHg). 25 healthy normotensive individuals were used as controls. Compared to controls, 24.6 mean BP was raised 15% in Very Mild EH and 29% in Mild-Moderate EH. Forearm and coronary Rmin was elevated by 49% and 54% in Very Mild EH and 75% and 144% in Mild-Moderate EH, as compared to control persons, respectively. Coronary flow reserve was decreased by 23% in Very Mild EH and by 29% in Mild-Moderate EH, respectively. Thus both coronary and forearm Rmin (P<0.01) and (P<0.01) were altered more than could be expected from the raised BP level in Very Mild EH. In contrast, SVRI and left ventricular mass increased proportionally to the increased BP (19% and 15%, Very Mild EH; 33% and 24%, Mild-Moderate EH). In conclusion, microvascular changes are present even in mild hypertension at a degree which hardly can be due solely to adaptation to the degree of BP rise or rise in peripheral resistance. Such changes may thus be more central to the pathogenesis than previously thought.

**EFFECTS OF ANTHYPERTENSIVE TREATMENT ON CAPILLARY RAREFACION IN ESSENTIAL HYPERTENSION**

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Significant capillary rarefaction (CR) occurs in individuals with sustained and borderline essential hypertension (EH) and also in normotensive subjects at high risk of developing EH. Rarefaction is correlated with mean wall to lumen ratio of resistance arteries, which is an independent predictor of cardiovascular events. The aim of this study was to evaluate the effects of treating hypertension on CR. Methods: We studied 19 subjects with untreated mild-to-moderate EH (mean age 45.5±2.9 yr, BP 161±100/55±30mmHg) before and after a median of 6 months of antihypertensive treatment. We used intravital video-microscopy to measure basal i.e. functional (BCD) and maximal i.e. structural (MCD) capillaries densities on the dorsum of the middle finger according to a well-validated protocol. Results: There was significant reduction in BCD as expected to 134±85/22 mmHg, P<0.0001. Mean BCD increased from 56.2±0.66mm2 to 62.2±0.60mm2 and p<0.0001 and MCD increased from 61.9±0.6 field to 66.2±0.0001. There was no significant correlation between the change in brachial BP (systolic, diastolic or mean) and the change in BCD or MCD. Calcium antagonists monotherapy was associated with a significant increase in functional but not MCD. The combination of an ACE inhibitor or ARB with a CCB appeared to exert more beneficial effects on CR while monotherapy with ARB or ACEI was more effective in improving CR than other antihypertensive agents. Conclusions: Treatment of EH can reverse CR. Further studies are required to provide direct evidence relating CR to outcomes and to evaluate effects of its reversal.

**NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) A NEW EARLY MARKER OF DEHYDRATION IN THE ELDERLY**

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Rationale: In elderly dehydrated patients (Age >80 years), the early detection and prevention of acute kidney injury (AKI) and acute heart failure (AHF) is important but difficult. The diagnostic and prognostic value of NGAL, an early biomarker of AKI, has not been evaluated in comparison to serum creatinine, which is a biomarker of Glomerular Filtration Rate (GFR). Methodology: A total of 60 consecutive high risk patients admitted 2009 in a CardiacGeriatric Unit, and were divided into 2groups according the presence of dehydration. We compared age, Creatinine (mg/L), BNP(cut-off 149 ng/mL) by triage test and DFG by MDRD (cut-off <0.7mL/min/1.73m2) (Table 1). Results: (Table 1): Our results show that the NGAL value is higher than the cut-off in dehydrated patients. In addition, our results indicate that the age and BNP value are not different between these groups. Perspectives: These results clearly show that the evaluation of the NGAL level would help clinicians to identify early dehydration with AKI risk in the elderly.
Table 1

<table>
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<tr>
<th>N</th>
<th>Mean Age</th>
<th>NGAL</th>
<th>Creatinine</th>
<th>MDRD</th>
<th>BNP</th>
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<tbody>
<tr>
<td>31</td>
<td>84.77</td>
<td>222.32 (±262.37)</td>
<td>18.19 (±6.66)</td>
<td>48.36</td>
<td>459.76</td>
</tr>
<tr>
<td>29</td>
<td>83.10</td>
<td>132.76 (±81.85)</td>
<td>14.72 (±6.13)</td>
<td>60.66</td>
<td>533.24</td>
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INTERPLAY OF VASOACTIVE PEPTIDE SIGNALLING IN PAIN SENSATION

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Signalling through the receptor for adrenomedullin and calcitonin gene-related peptide (CGRP) that are on a molar basis the most potent vasodilators known so far, is present in nociceptive fibres and released upon stimulation. Recently, it could be shown in rat mesenteric resistance arteries that calcitonin gene related peptide (CGRP) selectively relaxes contractile responses to endothelin-1 (ET-1) [Meens et al., J Pharmacol Exp Ther. 2009;331(1):87–95] and that the release of ET-1 from the endothelin receptor A (ET-A) requires CGRP signaling [Meens et al. Hypertension. 2009; 54(5):1186]. In addition ET-A stimulation evokes pain sensation. Mice overexpressing a smooth muscle α-actin promoter driven calcitonin receptor-like receptor (CL-α-tg) showed higher tolerance in fights for hierarchy suggesting reduced nociception. This could be confirmed by a reduced blood pressure and heart rate response as well as c-fos expression in dorsal horn neurons in response to noxious heat stimulation in anesthesia. In comparison to their corresponding wt controls, CL-α-tg showed significantly less and α-CGRP induced licking/biting behavior on the ET-1 injected hind paw. Pretreatment with α-CGRP reduced in all animals the licking/biting behavior on the ET-1 injected hind paw whereas it was increased when pre-treating the animals with the CGRP antagonist CGRP(8–37) in the same molar dosages as used for the CGRP pre-treatment. We suggest that CGRP released from nociceptive fibers upon ET-1 stimulation might act as a negative feedback mechanism to control the intensity of pain sensation.

PLATELET PRESERVATION DURING CARDIOPULMONARY BYPASS WITH APROTININ

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Purpose: Here we have investigated three novel recombinant aprotinin variants with specific modifications to the active site lysine at amino acid position 15 (arginine-15, arginine-15-alanine-17, and valine-15-leucine-17) for their effect on PAR1-mediated platelet aggregation in vitro. Methods: Aggregation studies were carried out using washed human platelets (n=9) or platelet rich plasma (n=7) from healthy volunteers activated with 1 or 5 nM thrombin. Recombinant aprotinin variants were used at the molar equivalent of 50 KIU/mL of the parent compound. Results: Platelet aggregation at low concentrations of thrombin (1 nM) was mediated exclusively through PAR1, as shown by inhibition of aggregation in the presence of FLLRN. At 5 nM thrombin, the mean percentage± SD aggregation of washed platelets was 68.6± 12.3%. This was suppressed by each aprotinin variant at the 50 KIU/mL equivalent dose: arginine-15-alanine-17 (33.3%: 22.9%, p<0.01); aprotinin (37.5%: 19.4%, p<0.05); valine-15-leucine-17 (50.0%: 16.1%, not significant). At 5 nM thrombin, which activates both high (PAR1) and low (PAR4) affinity (PAR4) thrombin receptors on platelets, FLLRN and aprotinin failed to block aggregation: this finding indicates that aprotinin selectively targets PAR1. In platelet-rich plasma, aggregation at 1 nM thrombin was 77.1%: 10.0%, and this was inhibited in the following order: arginine-15 (30.1%: 9.6%, p<0.001); arginine-15-alanine-17 (52.9%: 9.7%, p<0.001). Conclusion: We have found that the mechanism of action of aprotinin and its derivatives, safer and more efficacious aprotinin variants may become available for clinical use.

IN VITRO EVALUATION STENT WITH ENDOTHELIAL CELLS CONTAINING VEGF-GENE

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Vascular stent implanted in coronary artery is critical treatment to the coronary heart disease. But platelets adhesion and smooth muscle cells proliferation and migration aggravate restenosis, so thrombus formation and in-stent restenosis (ISR) are the major complication, endothelialization. We hypothesis that endothelialization may solve this problem. The VEGF gene was obtained by ScariI and DauII digestion after sequencing and then it was inserted into pIREs-EGFP vector to construct pIREs-EGFP-VEGF. The recombinant vector pIREs-EGFP-VEGF was transfected into HUVECs by lipofectamine 2000, and then the secretary of NO, cell cycle and VEGF expression of transgenic HUVECs was evaluated. The results suggested that VEGF gene transfection promoted the HUVEC proliferation, compared with control (p<0.05). VEGF, transfection could promote NO secretion and VEGF expression. Angiogenesis and migration of HUVEC was improved simultaneously but angiogenesis had not a qualitative difference compare to control. Proliferation of VSMC was not improved after stimulated by supernatant of transgenic cells and grow rate lower than control group at the fifth day. Transgenic endothelial cells were seeded onto the stent surface by rotary apparatus and these cells grew well with expressing green fluorescent protein. In our research recombinant vector pIREs-EGFP-VEGF transfected HUVEC could improve the expression of VEGF. Some detection on the transgenic cells provided in vitro support for animal experiments.

NOVEL VASCULAR GRAFT WITH HELICAL SLOT INHIBITS THROMBOSIS AND INTIMAL HYPERPLASIA

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Background: Small-diameter vascular grafts are in large demand for coronary and peripheral bypass procedures, but present products still fail in long-term clinical application. Based on the vascular anatomical characters, our hypothesis is that a novel graft with inside spiral slot would make the blood flow rotate to improve graft patency rate. Methods: Male or female beagles aged 1 to 1.5 years were used. An approximately 3–5 cm abdominal arterial replacement was made. In treatment group, the artery was replaced with spiral slot (8 mm in diameter) (n = 3); in control group, concentrically cramped (n = 3). The operative procedure was side-to-side anastomosis using 7–0 polypropylene sutures. graft patency and dimension were evaluated by computer tomography (CT) every month postoperatively. The animals were killed 6 months postoperatively and samples were evaluated histologically. Results: The grafts in all the dogs maintained patency for at least 24 weeks. In addition, CT revealed no dilatation in diameter or changes during the follow-up period. Macro examination of the spiral slot grafts showed no thrombus and they exhibited a smooth and glistening surface. Histological findings revealed complete reendothelialization, and normal smooth muscle layer had formed in the vascular wall. The control group has formed a thick smooth muscle layer and serious collagen localization, so has a much stenotic lumen. Conclusion: Spiral slot graft may change the velocity distribution near the internal surface, which could enhance graft patency rate, and is a promising way for vascular grafts to control thrombosis and intimal hyperplasia.

TIMELY RHYTHM ASSESSMENT FOR CORONARY EVENTS AT THE EMERGENCY ROOM OF THE PHILIPPINE GENERAL HOSPITAL (TRACER)

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The centrality of the electrocardiogram in the decision-making pathway for chest pain cannot be overemphasized. Consensus guidelines have established a 10-minute goal to first ECG for patients with acute chest pain or symptoms suggestive of myocardial ischemia. This study determined how effective the Philippine General Hospital is in delivering electrocardiographic services, primarily by computing lag time to first ECG at the emergency room (ER), as well as noting the patient profile and pattern of ECG utilization. Hospital records were reviewed for October 2007 and included adult patients presenting with chest pain. Of the 110 patients seen, 59% were male, and mostly aged 40–60 years. Chest pain consults peaked at 10AM to 2PM and 8PM to 12MN. Thirty one patients (28%) were either redirected to the outpatient department, sent home, went home against advice or transferred to another facility. Sixty-three patients (57%) had an ER ECG. Of the 85 patients considered to be at higher risk for ischemia, only 3 (3.5%) got their ECG within 15 minutes of admission. The average lag time to first ECG was 109 minutes, while the longest lag time was 643 minutes. The study revealed that: 1) many patients with chest pain did not benefit from an ER ECG, 2) for those who did, majority of them underwent the ECG too late, and 3) the process of triaging may have contributed to its under-utilization. This underscores the need to improve the quality of care in the institution.
**PD.07**

**PROTECTIVE EFFECT OF CAPTOPRIL AND ENALAPRILAT, ANGIOTENSIN-CONVERTING ENZYME INHIBITORS, ON PARA-NONYLPHENOL-INDUCED -OH GENERATION AND DOPAMINE EFFLUX IN RAT STRIATUM**

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We recently reported that para-nonylphenol, an environmental chemical, induced hydroxyl radical (·OH) formation in rat striatum. In this study we examined the antioxidative effects of angiotensin-converting enzyme inhibitors (captopril or enalaprilat) on para-nonylphenol and MPP+ (1-methyl-4-phenylpyridinium ion)-induced hydroxyl radical (·OH) formation and dopamine (DA) efflux in extracellular fluid of rat striatum, using a microdialysis technique. Para-nonylphenol clearly enhanced -OH formation and DA efflux induced by MPP+. When captopril or enalaprilat was infused in para-nonylphenol and MPP+ -treated rats, DA efflux and -OH formation significantly decreased, as compared with that in the para-nonylphenol and MPP+ -treated control. We compared the ability of non-SH-containing enalaprilat with a SH-containing captopril to scavenge ·OH and DA efflux. Both inhibitors were able to scavenge ·OH and DA efflux induced by para-nonylphenol and MPP+. The results suggest that angiotensin-converting enzyme inhibitors may protect against para-nonylphenol and MPP+ -induced -OH formation via suppressing DA efflux in the rat striatum.

**PD.08**

**EFFECT OF NON-PULSATILE EX VIVO PERFUSION WITH CRESCENT Pressures in Human Saphenous Veins (HSV): Morphological, immunohistochemical and biochemical study**

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**Background:** Exposure to mechanical forces causes HSV morphological and functional alterations. **Objectives:** To verify the HSV effects of non-pulsatile ex vivo perfusion with crescent pressures during three hours. **Materials and Methods:** Intact segments of HSV were obtained from 30 patients submitted to elective coronary artery bypass graft surgery. Ex vivo perfusion was performed during three hours, using oxygenated Krebs solution, flow of 100 ml/min and pressures of 0, 50, 100, 200 and 300 mmHg, defining five groups (n=6). After perfusion, the following analyses were performed: vein wall morphology, with optical and transmission electron microscopy; immunohistochemical expression of nitric oxide synthase (NOS) isoforms, CD34 and nitrotyrosine; tissue levels of nitrite/nitrate and malondialdehyde (MDA). **Results:** Optical microscopy showed that veins of groups perfused with 200 and 300 mmHg presented more developed endothelium, smaller adventitial processes and luminal slits; greater luminal area and; decreased the percentual of luminal perimeter covered by endothelium. Electron microscopy transmission showed alterations in veins perfused with 200 and 300 mmHg. Immunohistochemcal expression of the three NOS isoforms were observed in all vein layers, without significant difference among groups. CD34 Immunohistochemical expression, tissue levels of nitrate/nitrite were not significantly different among distinctive perfusion. Nitrotyrosine was not immunohistochemically expressed in all veins and MDA tissue levels were not different among groups. **Conclusions:** Non-pulsatile ex vivo perfusion during three hours caused morphological alterations in HSVs, which were not accompanied by immunohistochemical and biochemical alterations. Even with mechanical lesions, HSVs maintained the ability of expressing NOS and nitric oxide release.

**PE.01**

**VITAMIN D LEVELS AND MORTALITY IN TYPE 2 DIABETES**

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**Objective:** To evaluate vitamin D as predictor of all-cause and cardiovascular mortality and risk of progression to micro- or macroalbuminuria in type 2 diabetic patients. **Research Design and Methods:** In a longitudinal observational follow-up study, 295 type 2 diabetic patients with normoalbuminuria (n=172), microalbuminuria (n=73) and macroalbuminuria (n=44) at baseline, were followed for a median (interquartile range) of 10.0 (5.0-23.0) years. Mean (SD) age was 54(6) years. Plasma 25-hydroxyvitamin D3, 25(OH)D3, levels were determined by high performance liquid chromatography/tandem mass spectrometry on baseline samples. Severe vitamin D deficiency was defined as the lower 10% percentile (<13.9nmol/l). **Results:** Median (range) vitamin D level was 35.7 (5.1-136.7) nmol/l. Vitamin D levels were not associated with age, sex, estimated glomerular filtration rate (eGFR), urinary albumin excretion rate (UAER) or HbA1c at baseline, but low levels were weakly associated with elevated systolic blood pressure (R=0.13, p<0.03). During follow-up, 196 (88%) patients died. All-cause mortality was increased in patients with severe vitamin D deficiency; HR (95% CI) 1.96 [1.29-2.96]. The association persisted after adjustment for UAER, HbA1c, diabetes duration and conventional cardiovascular risk factors; HR 2.03 [1.31-3.13]. Severe vitamin D deficiency was associated with increased cardiovascular mortality; HR 1.95 [1.11-3.44]. The association persisted after adjustment; HR 1.90 [1.15-3.10]. Severe vitamin D deficiency at baseline did not predict progression to micro- or macroalbuminuria. **Conclusions:** In type 2 diabetic patients, severe vitamin D deficiency predicts increased risk of all-cause and cardiovascular mortality, independent of UAER and conventional cardiovascular risk factors. Whether vitamin D substitution improves prognosis remains to be investigated.

**PE.02**

**ROLE OF NOREPINEPHRINE TRANSPORTER IN BLOOD PRESSURE REGULATION, CARDIAC REMODELLING AND CIRCADIAN RHYTHM**

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The noradrenaline transporter (NET) is critical in central modulation of sympathetic tone and autonomic regulation of peripheral hemodynamics. It is crucial in limiting catecholaminergic signaling. NET removes not only residual noradrenaline (NE) but, a major portion of surplus dopamine as well. Peripherally, NET is most important in the heart. Using NET deficient mice, we characterized consequences of reduced NE rapture on hemodynamics (1), cardiac remodelling (2) and on circadian rhythm (3). Telemetrically we detected an elevated basal arterial blood pressure in NET-KO compared to heterozygous or wild type mice as well as a significantly higher increased blood pressure after Angiotensin II infusion (14 days). Further, echocardiographic analysis under basal conditions revealed in NET-KO mice a regular cardiac performance and architecture. However, after Angiotensin II administration for 14 days the NET-KO hearts showed a significantly aggravated left ventricular hypertrophy. To determine the impact of the noradrenergic signaling on circadian rhythm we telemetrically monitored the acrophases of blood pressure, heart rate and locomotor activity in WT, NET +/- and NET --/- under normal and variable day/night conditions. Basally, no differences in acrophases were detectable. However, the switch to a new day/night modus resulted in a retarded adjustment of NET-KO mice to the altered condition. In conclusion, our results show that Noradrenaline rapture by NET is involved in blood pressure regulation. NET also modulates the sensitivity to Angiotensin II. Further, we demonstrate that NET is involved in cardiac adaptation and remodeling after chronic stress. Finally we reveal NETs function in the regulation of circadian rhythm.

**PE.03**

**PARATHYROID HORMONE AND VITAMIN D STATUS IN CHRONIC HEART FAILURE (CHF)**

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**Background:** Hyperparathyroidism, hypovitaminosis D and disturbances of calcium-phosphorus metabolism are common in chronic kidney diseases, but they didn’t study well in CHF. **Methods:** 103 stable I-IV NYHA class CHF patients without primary renal, autoimmune, oncological, inflammatory bowel and diseases were included in study [median (interquartile range) of age 68.5(8.0–71.0) years; left ventricular ejection fraction (LVEF, Simpson) 36.3(27.6–39.9)]. We estimated serum intact PTH, phosphorus and calcium corrected for albumin, 25-OH vitamin D, urinary albumin excretion (UAEx), glomerular filtration rate (GFR, MDRD). **Results:** Hyperparathyroidism was revealed in 78.1%, hypovitaminosis D in 33.3%, hypercalcemia in 48.3%, hypercalcaemia in 3.4%, hyperphosphatemia in 10.3%, hyperphosphatemia in 6.8% of patients. Subjects with hyperparathyroidism compared to others had decreased LV EF [34.3(30.2–28.1) vs 41.3(36.4–44.9), p=0.038], GFR [69.5(56.7–85.9) vs 84.5(80.3–88.3) ml/min/1.73m2, p=0.003], elevated urinary albumin excretion (15.8(1–24) vs 5.6(1–7.6) mg/l, p=0.044) and prolonged CHF duration (60(52–69) vs 41(24–42) months, p=0.049). PTH correlated with vitamin D level (r=-0.5, p=0.01). PTH level wasn’t associated with age, serum calcium, phosphorus, NYHA class, splanholactone and furoside serum. Serum calcium correlated with LV end diastolic volume r=0.41, p=0.04. Serum phosphorus and calcium-phosphorus product correlated with UAE: r=0.6, p=0.007 and r=0.61, p=0.009. Cumulative proportion surviving was significantly less in group with calcium-phosphorus products higher median than 33.1(27.8–38.8) mg/dl2 (p=0.006, Cox’s F-test). After adjusting for age, weight, echocardiography parameters and GFR, elevated calcium-phosphorus product was associated with all-cause mortality. **Conclusion:** Hyperparathyroidism, hypovitaminosis D and disturbances of calcium-phosphorus metabolism are common, related with renal, cardiac dysfunction and poor prognosis in CHF.
HYPERCHOLESTEROLEMIA IMPAIRS THE MYOCARDIAL ANGIOGENIC RESPONSE IN A SWINE MODEL OF CHRONIC ISCHEMIA
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Purpose: We investigated the effects of hypercholesterolemia on the functional angiogenic response and collateral formation induced by chronic myocardial ischemia and the expression of angiogenic mediators. Methods: Twelve Yucatan miniswine, fed either a normal (NORM, n=6) or high cholesterol (HCHO, n=6) diet for 13 weeks, underwent amniotic constrictor placement around the circumflex artery. Three weeks later, myocardial perfusion was quantified using isotope-labeled microspheres. Seven weeks after amniotic placement, coronary microvascular responses and myocardial perfusion were assessed. Results: Coronary microvessels from HCHO pigs showed significant endothelial dysfunction. Baseline-adjusted myocardial flow at 7 weeks was significantly reduced in the HCHO animals (0.002±0.06 vs. 0.023±0.09 mL/min/g; HCHO versus NORM, p=0.04). Endostatin expression significantly increased in the HCHO pigs (2.2-fold, p<0.001). Endostatin expression was significantly lower in SHRs than in NORM pigs with moderate endothelial dysfunction (p<0.01). Conclusion: These findings suggest that under conditions of hypercholesterolemia, coronary collateral development may be regulated by endogenous angiogenesis inhibitors such as endostatin as well as reactive oxygen species.

CHRONIC MELATONIN TREATMENT REDUCES LV FIBROSIS AND DYSFUNCTION IN L-NAMEDIUede HTENSI6N AND IN SHR
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The pineal product and antioxidant, melatonin, reduces blood pressure (BP), enhances NO (nitric oxide) signaling and protects the myocardium against hypertrophic cardiomyopathy (HCM). The objective of this study was to evaluate the effect of chronic melatonin treatment on the progression of established HCM in SHR and the possible mechanisms involved. Methods: Male SHR were treated with melatonin (10 mg/kg/d) or vehicle (1% Tween 80, 0.9% saline) by intraperitoneal injection for 4 months. BP, left ventricular (LV) pressure, fractional area change (%FAC) and left ventricular mass were measured. Results: Melatonin significantly reduced BP, LV mass and LV pressure and improved %FAC. Histologically, LV interstitial fibrosis and number of hypertrophied cardiomyocytes were significantly lower in the melatonin treated group. Conclusion: Chronic treatment with melatonin improved LV mass, BP and myocardial function in SHR. These findings suggest a protective role of melatonin in the prevention of cardiomyopathy progression.

IN VITRO EVALUATION OF THE EXPRESSION OF DYSTROPHIN IN CARDIOMYOCYTES STIMULATED WITH SERUM OF MICE EXPERIMENTALLY-INFECTED WITH TYPHANOSOMA CRUZI
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Background: Dystrophin is implicated in the maintenance of the cell shape, mechanical resistance and signal transduction to cardiomyocytes. Results from our laboratory have shown decreased expression of the dystrophin glycoprotein complex (DGC), especially dystrophin, in experimentally-induced Typhanosoma cruzi myocarditis, both in the acute and chronic phase of the disease. This study tests the hypothesis that serum of mice experimentally-infected with T. cruzi affects the expression of dystrophin in cultured newborn cardiomyocytes. Methods: Cultured newborn cardiomyocytes were stimulated 5 days after their first spontaneous beating with serum of mice infected with T. cruzi for 24 hours. Serum was obtained from male C57Bl/6 mice infected with T. cruzi in the peak of cardiac tissue inflammation. Immunofluorescence (IF) staining and Western blotting (WB) were performed for evaluation of the expression of dystrophin, phalloidin, troponin, TNF-α, calpain, iNOS, and NF-κB. Results: The IF for dystrophin and phalloidin showed a decrease in expression of dystrophin and phalloidin and an increase in expression of iNOS and NF-κB. Western blotting showed that the expression of TNF-α, calpain, iNOS, and NF-κB was increased in the cells. Conclusions: Our results lend support to the hypothesis that serum of T. cruzi infected mice directly affects expression of dystrophin. The decrease of dystrophin expression may result in loss of dystrophin function and eventual cell death. This is a potential mechanism by which T. cruzi pathogenesis affects the myocardium.

DISRUPTION OF DYSTROPHIN AND β-DYSTROGLYCAN MAY BE A POTENTIAL MECHANISM FOR MYOCARDIAL DYSFUNCTION IN SEVERE SEPSIS
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Evidence from our laboratory has demonstrated alterations in myocardial structure in severe sepsis/septic shock. The morphological alterations are heralded by sarcolemmal damage characterized by increased membrane permeability caused by oxidative damage to lipids and proteins. The critical importance of the dystrophin-glycoprotein complex (DGC) in maintaining sarcolemmal stability led us to hypothesize that dystrophin loss and associated glycoproteins could be involved in early increased sarcolemmal permeability in experimentally induced septic cardiomyopathy. Male C57Bl/6 mice were subjected to sham operation and moderate (MSI) or severe (SSI) septic injury induced by cecal ligation and puncture (CLP). Using Western blot and Immunofluorescence, a down regulation of dystrophin and β-dystroglycan expression in both severe and moderate injury could be seen in septic hearts. Immunofluorescence and protein amount expressions of laminin-α2 may be altered in SSI and sham-operated hearts. Consonantly, the evaluation of plasma membrane permeability by intracellular albumin staining provided evidence of severe injury of the sarcolema in SSI hearts whereas antioxidant treatment significantly attenuated the sarcolemmal dystrophin loss expression and the increased membrane permeability. The main finding was that severe sepsis leads to a marked reduction in membrane localization of dystrophin and β-dystroglycan in septic cardiomyocytes, a process that may constitute a structural basis of sepsis-induced cardiac depression. In addition, increased sarcolemmal permeability suggests functional impairment of the DGC complex in cardiac myofibers. Finally, in vivo observation that antioxidant treatment significantly abrogated the loss of dystrophin expression and plasma membrane increased permeability suggests the hypothesis that oxidative damage may mediate the loss of dystrophin and β-dystroglycan in septic mice.
PE.09 ACUTE TREATMENT OF COPPER TOXICITY IN EX VIVO PERFUSED HEART MODEL AND IN VITRO CARDIOMYOCYTES MODEL WITH TRIETHYLENETETRAMINE
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Recent studies have shown that chronic treatment with a divalent Cu^2+ selective chelator (triethylenetetramine) can alleviate heart failure, improves left ventricular hypertrophy and increase urinary Cu excretion in diabetic rat and human. In this study, we examine the role of triethylenetetramine as a potential acute treatment for copper toxicity in perfused heart and using cardiomyocytes to model the molecular changes in response to copper overload. Cupric chloride (CuCl) solution was infused into isolated rat hearts (male Wistar rat), followed by the infusion of triethylenetetramine solution or control buffer. Differentiated cardiomyocytes were cultured with different concentrations of CuCl in the presence or absence of relevant triethylenetetramine dihydrolchloride concentrations. Our data indicated that short term perfusion of CuCl to ex vivo isolated heart can immediately impair cardiac function in a dose dependent manner. With triethylenetetramine treatment, the cardiac function of the copper perfused heart can be partially restored. Furthermore, the effect of triethylenetetramine treatment is copper-specific as no effect was observed in cardiomyocytes treated with different concentrations of CuCl in the presence or absence of relevant triethylenetetramine dihydrolchloride concentrations. In cultured cardiomyocytes study, we found that the mRNA expression of metallothioneins II (MT2) was significantly increased following addition of CuCl to the culture medium, indicating an important role of MT2 in protection against acute copper toxicity. In conclusion, our study has demonstrated a copper toxicity effect in both in vivo and in vitro model, and provides evidence that triethylenetetramine may act as a potential acute treatment for copper toxicity in the heart.

PE.10 COMPARISON BETWEEN CARDIAC HYPERTROPHY INDUCED BY EXERCISE TRAINING AND LONG TERM INTAKE OF NIGELLA SATIVA
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Background: Exercise training is employed nowadays for the treatment of heart failure, as it induces physiological cardiac hypertrophy. Similarly, Nigella sativa (NS) is found to induce cardiac hypertrophy and enhance cardiac function. NS-cardiac hypertrophy might be a physiological hypertrophy. Therefore, we aim to study NS-induced cardiac hypertrophy, and compare it to exercise-induced cardiac hypertrophy. Methods: Sixty Wistar rats were divided into: control, NS, exercise and NS-exercise groups. Daily 800mg/Kg NS was administered orally to NS and NS-exercise rats for 8 weeks. Exercise and NS-exercise rats ran on treadmill, 2 hour/day for 8weeks. By the end of the experiment, ECG, body (BW), heart (HW) and left ventricular weights (LVW) were recorded. H&E and PAS sections were prepared to study the histology of left ventricular wall and to measure diameters of cardiomyocytes. Results: HW/BW, LW/BW and mean diameter of cardiomyocytes were significantly higher in all experimental groups than the control. Histology showed normal cardiomyocytes and no excess fibrosis in any of the studied specimens. Heart rate was significantly lower, ORG amplitude and specific potential (amplitude/LWV) were significantly higher in NS-exercise group than the control. Conclusion: NS-induced cardiac hypertrophy is evidenced by significantly higher HW/BW, LW/BW and larger diameter of cardiomyocytes. NS demonstrated a synergistic effect with exercise training as NS-exercise induced cardiac hypertrophy had lower heart rate, and well matched electrical activity of the heart to its mass. Therefore, this model of cardiac hypertrophy might be introduced as a new therapeutic strategy for treatment of heart failure with superior advantages to exercise training.

PE.11 THE EFFECT OF DEFERASIROX ON TISSUE DOPPLER ECOCARDIOGRAPHIC INDICES IN PATIENTS WITH BETA-THALASSEMIA MAJOR
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We examined the effects of low and high doses of the oral chelating therapy deferasirox for six months on pulsed and tissue echocardiographic parameters in patients with beta-thalassemia major (β-TM) in Bahrain. The study include a control healthy group (n=32), 2) β-TM patients treated with deferasirox 20 mg/kg/day (n=38), and 3) β-TM patients treated with deferasirox 40 mg/kg/day (n=28). All study groups underwent pulsed Doppler echocardiography and biochemical analysis of serum alanine transaminase (ALT), creatinine, ferritin and plasma pro-BNP. Compared with controls, both groups of β-TM patients had thicker indices of LV septal and posterior walls (P<0.01), higher LVEDD index (P<0.05), higher LV transmural E-wave velocity (P= 0.03) and E/A ratio (P<0.01), and shorter deceleration time (DT) and IVRT (P<0.01). The ratio of transmural E wave velocity to the E wave at the basal septal mitral annulus (E/E′m) was significantly higher in β-TM group (P<0.01). Despite the significantly lower serum ferritin in patients treated with high dose of deferasirox (P<0.001) compared with those on low dose, there were no significant differences in tissue Doppler indices, plasma pro-BNP, serum ALT or creatinine. We confirm our previous findings that left ventricle diastolic indices measured by tissue Doppler echocardiography are compromised in patients with β-thalassemia major. We add in this study that using a dose of 40 mg deferasirox for 6 months significantly reduced serum ferritin, but did not improve the ventricular diastolic indices measured by tissue Doppler or by measuring plasma BNP.

PF.01 KNOCKOUT OF α-LP (2A)-ADRENERGIC RECEPTOR AMELIORATES PROGRESSION OF CHRONIC KIDNEY DISEASE
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Chronic kidney disease (CKD) is a major health issue. Investigations were carried out to analyse the function of α2A-adrenergic receptor (AR) for progression of CKD. The α2AR is known as main regulator of presynaptic norepinephrine release. A murine knockout model (KO) with deletion of alpha2A-AR was used and compared to its wild-type (WT). In kidneys of KO mice presynaptic neurotransmission after renal nerve stimulation was significantly higher than in WT mice. The hypothesis that this increased sympathetic tone in KO mice accelerates progression of chronic kidney disease was tested inducing experimental renal failure by subtotal nephrectomy (SNX). After challenge (SNX) WT and KO mice developed albuminuria which was significant higher in WT mice. Kaplan-Meier survival analysis revealed a diminished mortality of KO mice. 60 days after SNX, initial group size of n=12 was reduced to n=5 in WT mice and n=3 in KO mice. Results suggested a major role of postnephrectomically located α2A-adrenergic receptor in this respect. To illuminate postnephrectomy effects cell culture model was used. HEK 293T cells were transfected with α2A-AR and stimulated with the selective α2A-agonist UK14,304. Stimulation of α2A-HEK293T-cells induced a concentration- and time-dependent phosporylation of extracellular signal-regulated-kinases ERK1/2. In conclusion, the examinations suggest a major role of postnephrectomically located α2A-adrenergic receptor, which seems to be a point of action for catecholamines regulating mitogen-activated protein (MAP) kinases pathways and possibly accelerate fibrotic processes in CKD.

PF.02 GLOMERULAR ENDOTHELIAL CELLS EXPRESS THE PROINFLAMMATORY CYTOKINE INTERLEUKIN-17 IN ACUTE KIDNEY DISEASES
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Overexpression of pro-fibrotic and pro-inflammatory cytokines and extracellular matrix accumulation are hallmarks of acute glomerular injury. A connection between TGF-β signaling and the inflammatory cytokine IL-17, as well as IL-17 expression by renal cells in kidney diseases has recently been proposed. The present study investigated IL-17 expression in different glomerular cell types in rat model of acute anti-Thy1 glomerulonephritis (aGN). aGN was induced in male Wistar rats by i.v. injection of anti-Thy1 OX-7 antibody. Tissues were harvested at the day 5 (matrix expansion), PBS-injected animals served as controls (con). Immunofluorescence was performed for cell marker (OX-7: mesangial cells, PECAM: endothelial cells, synaptopodin: podocytes) and IL-17. For in vitro experiments NRK 52E cells were used. Induction of aGN was characterized by marked proteinuria (di 113±12 mg/d; 3.9-fold vs. con; p<0.001) and histological glomerular-matrix accumulation (di 5±3 fold vs. con; p<0.001), in parallel with highest TGF-β and IL-17 mRNA expression (2.25-fold; + 6.5-fold vs. con; p<0.05). Immunofluorescence staining with synaptopodin and OX-7 showed no colocalization with IL-17, whereas PECAM was highly colocalized with IL-17. In vitro, IL-17 was secreted by NRK 52E at basal levels, up-regulated after exposure to 25 mM glucose (+ 7-fold) and reversed in the presence of a specific TGF-β receptor blocker (SB 431542). Stimulation of cells with TGF-β or IL-6 amplified IL-17 expression by 2.0-fold. Co-administration of TGF-β and IL-6 led to highly synergistically enhanced IL-17 secretion by more than 4000-fold. This study documents the expression of the new pro-inflammatory cytokine IL-17 by glomerular endothelial cells in acute, anti-Thy1 glomerulonephritis, thus under pro-fibrotic and pro-inflammatory conditions.

PF.03 INTERLEUKIN 17 - A NEW ACTOR IN ACUTE AND CHRONIC KIDNEY INJURY
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Overexpression of pro-fibrotic and pro-inflammatory cytokines, such as TGF-β and IL-6, and extracellular matrix accumulation are hallmarks of acute and chronic glomerular injury. A connection between TGF-β signaling and the inflammatory cytokine IL-17 has recently been proposed. This study analyzed the expression patterns of TGF-β and IL-17 in rat models of acute anti-Thy1 glomerulonephritis (aGN), in streptozotocin-induced 

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PF.04

INDUCTION OF SPONTANEOUS ALBUMINURIA IN SHR RATS BY TRANSFER OF A GENETIC LOCUS FROM THE MUNICH WISTAR FRÖMTER RAT

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Albuminuria is a polygenic quantitative trait indicating target organ damage and increased cardiovascular risk. Inbred Munich Wistar Frömter (MWF) rats develop significant albuminuria that is under polygenic influence, while spontaneously hypertensive rats (SHR) are resistant to albuminuria development. We previously identified a major quantitative trait locus (QTL) on rat chromosome (RNO8) for albuminuria in MWF. In contrast to the common protocol in which a QTL is replaced in the disease strain by the allele of the normal strain, in this study we tested if a consomic SHR-8MWF strain can be resistant to albuminuria development. For this purpose, we generated a consomic SHR-8MWF strain by transferring RNO8 from the normal strain in the SHR. We show that this strain is resistant to albuminuria development. We previously identified a major quantitative trait locus (QTL) on rat chromosome (RNO8) for albuminuria in MWF. In contrast to the common protocol in which a QTL is replaced in the disease strain by the allele of the normal strain, in this study we tested if a consomic SHR-8MWF strain can be resistant to albuminuria development. For this purpose, we generated a consomic SHR-8MWF strain by transferring RNO8 from the normal strain in the SHR. We show that this strain is resistant to albuminuria development. We previously identified a major quantitative trait locus (QTL) on rat chromosome (RNO8) for albuminuria in MWF. In contrast to the common protocol in which a QTL is replaced in the disease strain by the allele of the normal strain, in this study we tested if a consomic SHR-8MWF strain can be resistant to albuminuria development. For this purpose, we generated a consomic SHR-8MWF strain by transferring RNO8 from the normal strain in the SHR. We show that this strain is resistant to albuminuria development. We previously identified a major quantitative trait locus (QTL) on rat chromosome (RNO8) for albuminuria in MWF. In contrast to the common protocol in which a QTL is replaced in the disease strain by the allele of the normal strain, in this study we tested if a consomic SHR-8MWF strain can be resistant to albuminuria development. For this purpose, we generated a consomic SHR-8MWF strain by transferring RNO8 from the normal strain in the SHR. We show that this strain is resistant to albuminuria development.

PF.05

TELMISARTAN IMPROVES METABOLIC PROFILE IN OBESE PATIENTS WITH ARTERIAL HYPERTENSION

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Background: There are several lines of evidence suggesting that telmisartan may improve metabolic profile. The aim of the study was to estimate changes of insulin resistance and plasma level of adiponectin after long-term antihypertensive treatment with telmisartan in obese hypertensive patients. Patients and methods: Thirty four previously untreated obese adults with arterial hypertension were enrolled into the study. Euglycemic-hyperinsulinemic clamp technique was applied for measurement of glucose cells uptake (M) and calculation of M to insulin plasma concentration ratio (M/I). M and M/I values, body fat content (by DXA method), as well as plasma concentration of adiponectin, its high molecular weight fraction and IL-17 and TGF-β mRNA-expression were measured at baseline and after 2, 4, 6 months of the therapy, and two months after treatment cessation. Results: Treatment with telmisartan resulted in 21.9% and 30.1% decrease of body fat content and plasma level of adiponectin, respectively. The study documents a time-dependent expression of the new pro-inflammatory cytokine IL-17 in acute anti-thy1 glomerulonephritis. In SHR, HK and cGs rats, IL-17 and TGF-β are co-expressed by glomerular cells.

PF.06

THE INFLUENCE OF ATORVASTATIN THERAPY ON PLASMA CONCENTRATIONS OF ADIPONECTIN, RESISTIN AND LEPTIN IN PATIENTS WITH METABOLIC SYNDROME

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Background: Disturbed production of adipokines by visceral fat tissue seems to play a key role in the pathogenesis of metabolic syndrome. However, it has not been undoubtedly proven whether or not, such a beneficial effect of statins on cardiovascular system is mediated through their influence on secretion of adipokines. Therefore the aim of this single center, prospective, open label study was to assess the influence of 6-months atorvastatin (Sortis) therapy on plasma adiponectin, resistin and leptin concentrations in patients with metabolic syndrome. Material and methods: Thirty six adult patients with metabolic syndrome and serum total cholesterol over 200mg/dl, previously untreated with any statin were included into the study. All patients received 10mg of atorvastatin for 6 months. Plasma concentrations of insulin, adiponectin, leptin and resistin were measured before initiation, after 2, 4, 6 months of the therapy, and two months after treatment cessation. Results: Treatment with telmisartan was followed by 35.6% decline of LDL of cholesterol. Plasma adiponectin concentration decreased by 20.7% after 2 months of atorvastatin treatment. However, later this effects was declining and after 6 months plasma adiponectin concentration did not differ significantly from the initial values. Treatement with atorvastatin did not influence significantly either plasma leptin or resistin concentrations. Conclusion: Transient and moderate decrease of plasma adiponectin concentration without concomitant changes of leptin and resistin levels is observed during atorvastatin therapy.

PF.07

RAAS STIMULATION AT YOUNG AGE CAUSES SUSTAINED HYPERTENSION: A STORY OF IRREVERSIBLE RENAL DAMAGE?

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We tested if transient stimulation of the renin angiotensin aldosterone system (RAAS) at young age would permanently increase blood pressure (BP). In particular, we investigated the role of the kidney in this process. Studies were performed in Cyp1a1 Ren2 transgenic rats with inducible hypertension. These rats harbor a construct for the production of mouse renin which becomes activated when 0.3% indole-3-carbinol (IC3) is added to the diet. Experimental rats received IC3-treatment between 4–8 weeks of age (iwo) or between 30–34 iwo. Non-treated rats served as control. BP follow-up was performed via taillum and intra-arterial BP was determined at 8, 12, 20 iwo and 34 and 33 iwo. Additionally, renal vascular resistance and pathology were determined. Data are presented as mean ± SEM. At 20 iwo, i.e. 12 weeks after IC3 withdrawal, IC3-treated rats still demonstrate a statistically significant increase in BP compared with controls (141±7 versus 125±6 mmHg). In adult rats, BP remains immediately to control values after stopping renin activation. PAS-D staining on renal sections reveals a sustained increase in Glomerular Index (GI) and Tubulointerstitial Score (TIS) for the young rats. Adult rats also demonstrate an increase in GFR and TIS upon IC3-treatment, however this damage is disappeared 4 weeks later, i.e. at 38 iwo. This study shows that, at least in rats, sustained hypertension can be induced by a transient stimulation of the RAAS in an early stage of life with a crucial role for renal damage.

PF.08

RELATION OF RENAL FUNCTION, MICRALBUMINURIA AND RENAL BLOOD FLOW WITH ATRIAL FIBRILLATION IN CHRONIC HEART FAILURE (CHF)

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Background: Renal dysfunction increased the risk of new onset of atrial fibrillation (AF), and AF increased the risk of kidney disease in community. Renal dysfunction is widespread and associated with poor prognosis in CHF. Our aim was to estimate relationship between renal function, microalbuminuria, renal blood flow (RBF) and AF in CHF. Methods: In 70 stable CHF patients I-IV NYHA class without primary renal, renal vessel, endocrine, oncological diseases [median (interquartile range) of age 61.5(54.0 – 70.0) years; left ventricular ejection fraction (Simpson) 28.9(23.6 – 34.9), 92.9% males] we estimated AF prevalence, glomerular filtration rate (GFR, MDRD), urine albumin-creatinine ratio (ACR, immunoenzymatic assay), RBF (Duplex ultrasonography). Results: 35.7% (95%CI 32.7 – 48.2) of patients had permanent AF, others – sinus rhythm (GR, 12.9% had paroxysmal AF with last paroxysm in 6 > months ago). GR was lower [98.9(5.2 – 103.9) vs 102.7(97.8 – 108.2) ml/min/1.73m2, p = 0.042] and ACR was higher [32.7(9.1 – 46.3) vs 9.3(1.8 – 33.3) mg/g, p = 0.014] in AF in comparison with SR patients. RBF was decreased in AF (index of blood volume entering into the renal artery during single cardiac cycle was 1.7(1.2 – 2.3) vs 2.4(1.7 – 3.3) ml/m2, p = 0.015 for basal RBF,
2.7(2.2–3.7) vs 3.7(3.1–5.1) ml/m2, p = 0.005 for general RBF). We followed patients during 61 month. All-cause mortality was 49.3% (95%CI 35.9–62.7). AF didn’t affect the mortality (p > 0.05). But GFR decreased during follow-up time (p = 0.009) especially in AF vs SR patients (29.2(17.1–39.4) vs 9.8(3.5–13.8) ml/min/1.73m2, p = 0.019). **Conclusion:** CHF patients with atrial fibrillation are more at risk for the development of decreasing of GFR, RBF and microalbuminuria than those with sinus rhythm.

**Conclusion:**

CHF patients with atrial fibrillation are more at risk for the development of decreasing of GFR, RBF and microalbuminuria than those with sinus rhythm.

**MODEL ESTABLISHMENT OF LIPOPROTEIN ACCUMULATION OBSERVATION IN VIVO IN ZEBRAFISH**

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Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality in worldwide. Atherosclerotic lesions in humans develop preferentially at certain sites such as the curves, bifurcations and the straitness sites of arteries. Nevertheless, it has been well documented that early event leading to the genesis of atherosclerosis is the accumulation of cholesterol and other lipids within the intima. But it is few to investigate the relations between the lipoprotein accumulation and atherogenesis in vivo. We consider that the zebrafish is a wonderful model to investigate the lipoprotein accumulation and atherogenesis. The transgenic zebrafish (Fk1:mCherryRas) was used in this research. Injecting lipoprotein with green fluorescence into optically transparent zebrafish embryos (Day2, n = 60). By using confocal microscopy , the distribution of green fluorescent lipoprotein was observed immediately, and then the lipoprotein accumulation was observed every night (Day 2-Day 5). There was no fluorescent granule flowing in the vessel luminal in control group, meanwhile, the green fluorescent lipoprotein was flowing in the vessel luminal of the injection group (57/60) from the movies. Furthermore, the lipoprotein accumulation occurred after injection. Interestingly, the accumulation of lipoprotein was only uptake by the vein in zebrafish embryo. These results suggest that it is viable to inject fluorescent lipoprotein into zebrafish to study important characteristics of lipoprotein in the early atherogenesis. Therefore, zebrafish is a proper model to investigate the lipoprotein accumulation and atherogenesis.