402. Pulmonary circulation: basic mechanisms, animal models and experimental treatments

P3897

Mir-17 modulates smooth muscle cell markers, apoptosis and BMPR-II levels in human pulmonary artery smooth muscle cells

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Pulmonary Arterial Hypertension (PAH) is a progressively devastating disease characterized by excessive proliferation of the Pulmonary Arterial Smooth Muscle Cells (PASMCs). Recently micro RNA (miR) have been shown to play an important role in the pathogenesis of PAH. We describe in the present study the effects of over expression of miR17/92 as a cluster or miR17 alone on human pulmonary artery smooth muscle cells (HPASMC). HPASMC were commercially obtained and were transfected with miR17/92 or miR17 encoding plasmid or control vector by electroporation. Proliferation and apoptosis resistant state of PASMC transfected with miR was assessed by MTS and caspase3/7 Glo assays respectively. RNA and protein levels of important target genes in PAH were measured using Real-time RT-PCR and western blots. HPASMC transfected with miR17/92 or miR17 show decreased cell numbers and showed an increased apoptosis as assessed by

increased caspase activity. Real-time RT-PCR analysis reveal that proliferation markers such as PCNA and Cyclin D1 are not significantly altered. SMC marker calponin and bone morphogenetic protein receptor-II levels were down regulated in both miR17/92 or mir17 transfected cells. Voltage gated potassium channel (Kv1.5) was up regulated in PASMCS treated with mir17/92 cluster but not miR17 alone indicating that different miRs in the 17/92 cluster differentially may regulate key molecules in the development of PAH.

P3898

Vasopressin is involved in endothelin receptor antagonist-induced fluid retention in rat. Differential effect of selective ETA and dual ETA/ETB receptor antagonists

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Endothelin receptor antagonists (ERAs) are associated with varying degrees of fluid retention. As endothelin B (ETB) receptors have been involved in natriuresis and diuresis, it was paradoxical to observe that ETA-selective ERAs cause a significant risk of fluid overload in patients.

Aim of the present study was to understand the contribution of each ET receptor subtype in the mechanism of fluid retention in rats. Changes in fluid balance were assessed after administration of the prototypic ETA-selective sitaxentan and the dual ETA/ETB receptor antagonist bosentan, by measuring haematocrit (Hct), haemoglobin (Hb), plasma volume (PV), body fluid content and renal excretory function.

Acutely, sitaxentan caused marked dose-dependent decreases in Hct and Hb, whereas bosentan had a lesser effect. Chronic studies confirmed this difference and showed that sitaxentan increased PV (+50%) and elevated total body fluid content (+15%) compared with vehicle, while bosentan had a small non-significant effect (+16% for PV and +8% for total body water content). In addition, sitaxentan, but not bosentan, reduced water excretion and increased plasma vasopressin (AVP) concentration (3-fold increase) compared with vehicle-treated rats. In Brattleboro rats lacking AVP and in Wistar rats treated with either the AVP V2 receptor antagonist tolvaptan, or the ETB-selective antagonist BQ-788, hemodilution induced by sitaxentan was markedly reduced.

These results demonstrate that ERAs, particularly ETA-selective antagonists cause fluid retention by activating the AVP system via secondary stimulation by endothelin of the uninhibited ETB receptors.

P3899

A potential new therapy for pulmonary hypertension with the use of compound 21, an angiotensin type 2 receptor (AT2R) agonist

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Pulmonary hypertension (PH) is a debilitating, chronic lung disease that often leads to right-heart failure and death. Currently available therapies are ineffective in significantly improving the quality of life and reducing mortality rates, thereby necessitating the discovery of novel therapeutic interventions. The renin-angiotensin system (RAS) has been associated with the pathophysiology of PH, via increased activity of the deleterious ACE/ANGII/AT1R axis. It has been suggested that AT2R are upregulated in response to cardiovascular injury, and subsequent stimulation of this receptor may oppose the deleterious actions of the RAS axis. However, the role of AT2R in PH has yet to be investigated. We propose that non-peptide AT2R agonist, Compound-21 (C21), will attenuate the progression of monocrotaline (MCT)-induced PH in 8-week-old Sprague Dawley rats. Four weeks post a subcutaneous injection of MCT (50mg/kg), the rats displayed marked elevation in right ventricular systolic pressure (RVSP, Control: 31.5±0.78; MCT: 82.2±4.52mmHg; $p<0.05$; n=8-10), with subsequent development of right ventricular hypertrophy (RVH, Control: 0.25±0.005; MCT: 0.61±0.04; $p<0.05$; n=8-10). C21 treatment (0.03mg/kg/day i.p.) begun 2 weeks post MCT-challenge resulted in significant attenuation of RVSP and RVH increases (RVSP, MCT+C21: 64.2±5.62mmHg; RVH, MCT+C21: 0.48±0.04g; $p<0.05$; n=14). Furthermore, C21 significantly attenuated PH-induced right heart dysfunction, in terms of elevated right ventricular end-diastolic pressure and +dP/dt. Our results suggest that AT2R represents a novel therapeutic target in the management of PH, and C21 may serve as a lead beneficial compound.

P3900

A dose-response study of nilotinib and imatinib in experimental pulmonary hypertension

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Introduction: Platelet derived growth factor (PDGF) and c-kit are involved in the pathophysiology of pulmonary hypertension (PH). Tyrosine kinase inhibitor (TKI) targeting PDGF receptors and c-kit such as imatinib (Im) and nilotinib (Nil) are currently tested in PH.

Aims and objectives: To test the efficacy of Nil and Im in experimental PH.

Methods: Sprague-Dawley rats were analyzed, corresponding to controls (Cont), animals exposed to MCT, MCT and treated with Im at 50 or 100 mg/kg/j (MCT+Im50-MCT+Im100), MCT and treated with Nil at 40, 80 or 120 mg/kg/j (MCT+Nil40-MCT+Nil80-MCT+Nil120). TKI were administered from day 21 to 35 after MCT. Serum kinetics concentrations (SKC) of TKI were performed at day 28. At day 35 hemodynamic parameters, right cardiac hypertrophy and pulmonary vascular remodelling were studied.

Results: SCK showed that Im50,Nil40 and Nil80 corresponded to human drug concentrations. A dose-response improvement in hemodynamic parameters and medial wall thickness was observed with Im and Nil.

Conclusion: Dose-dependent improvements of experimental PH are observed with Nil and Im.

P3901

Genistein rescues pulmonary hypertension and attenuates abnormal vasoconstriction in rats lungs

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Background: Recent studies suggest that the deregulation of endothelial nitric oxide synthase (eNOS) is accountable for in the development of pulmonary hypertension (PH). Genistein, a phytoestrogen derived from soybean, has been reported to improve endothelial function.

Objective: We hypothesized that chronic treatment with genistein would prevent and reverse of hypoxic PH (HX) by improvement of eNOS function.

Method: Daily treatment with either genistein (0.2mg/kg) or vehicle was started. After 3-wk hypoxic exposure, rats underwent cardiac catheterization, examination of right ventricular hypertrophy, morphological features, Western blot analysis, and immunoprecipitation were performed. For evaluation of the reversal effect, genistein treatment started after rats had been exposed to 3-wk hypoxia exposure, and were examined in the similar way as described above. Furthermore, we examined if genistein would attenuate abnormal vasoconstriction in HX by isolatetd perfused lung examination.

Results: Genistein treatment prevented the progression of PH to right ventricular failure and restored vascular remodeling in HX. And also, genistein rescued pre-existing PH. These effects were mediated by improvement of eNOS function and restoring the interaction of eNOS and eNOS-related proteins.Furthermore, exogenously administration of genistein rapidly attenuated abnormal vasoconstriction in HX by improvement of eNOS function.

Conclusion: These results indicated that genistein not only had protective and reversal effects against the development of hypoxic PH, but also attenuated abnormal vasoconstriction of PH. The underlying mechanism might be related to the improvement of eNOS function.

P3902

Modified exhaled nitric oxide measurement in monocrotaline-exposed rats to monitor pulmonary hypertension

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Background and aims: Rats exposed to monocrotaline (MCT) are broadly used as animal model for pulmonary arterial hypertension (PAH). In analogy to human disease, right heart catheter (RHC) is the established gold standard for pulmonary pressure monitoring. Although exhaled nitric oxide (ENO) levels have been shown to correlate with pulmonary pressures in humans with pulmonary vascular disease, no link between ENO and pulmonary pressures could be established in rats. The

Abstract P3900 – Table 1

	Cont	MCT	MCT+Im50	MCT+Im100	MCT+Nil40	MCT+Nil80	MCT+Nil 120
RVSP (mmHg)	36,4±1,5	112,9±3,5**	96,0±6,1	85,3±8,43$^{\#}$	98,5±7,6	83,6±7,9$^{\#\#}$	83,8±6,0$^{\#\#}$
mPAP (mmHg)	14,7±0,7	47,5±0,9**	40,2±3,4	34,2±4,1$^{\#}$	42,7±3,1	35,0±3,0$^{\#}$	33,9±2,6$^{\#\#}$
CO (mL/min)	110,9±5,5	48,4±4,5**	89,8±6,9$^{\#\#}$	93,6±6,5$^{\#\#}$	88,2±8,3$^{\#\#}$	89,9±3,7$^{\#\#\#}$	93,4±6,4$^{\#\#\#}$
Fulton index	0,28±0,012	0,76±0,038***	0,59±0,037$^{\#\#}$	0,50±0,030$^{\#\#}$	0,67±00,068	0,55±0,031$^{\#\#}$	0,62±0,042$^{\#}$
Medial wall thickness	9.6±0.4	15.2±0.7***	12.2±0.5$^{\#}$	10.8±0.4$^{\#\#\#}$	11.4±0.4$^{\#\#\#}$	11.5±0.4$^{\#\#\#}$	10.7±0.4$^{\#\#\#}$

RVSP: Right ventricular systolic pressure, mPAP: mean pulmonary arterial pressure, CO: cardiac output. $^{\#}p<0.05$, $^{\#\#}p<0.005$, $^{\#\#\#}p<0.001$ vs MCT.

aim of the present study was to test whether a technical modification of the NO measurement process could help generate reliable ENO values that correlate with pulmonary pressures as assessed by simultaneous RHC.
Methods: 33 male Sprague-Dawley rats were studied 28 days after MCT-exposure and unilateral pneumonectomy. Hemodynamic parameters were monitored by an implantable telemetry system (DSI Datascience, St. Paul, MN U.S.A.). ENO was measured by means of chemiluminescence (CLD 66, Eco Physics, Duernten, Switzerland) in single, conscious, spontaneously breathing rats. ENO values measured by a standard accumulation method (ENO standard) and those obtained after process modification (ENO modified) were correlated with mean pulmonary arterial pressures (mPAP).
Results: After process modification, measuring errors were diminished and potential influencing factors eliminated. There was a clear correlation between ENO modified and mPAP (p=0.007; R=-.459), while no correlation was observed between ENO standard and mPAP (p=0.236; R=-.212).
Conclusion: Modified non-invasive ENO measurement may be used to monitor PAH in monocrotaline-exposed rats.

P3903
Bone morphogenetic protein signaling in experimental nitrofen-induced congenital diaphragmatic hernia
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Congenital diaphragmatic hernia (CDH) is a life-threatening cause of lung hypoplasia and persistent pulmonary hypertension of the newborn. As bone morphogenetic proteins (BMP) have been shown to play crucial roles in fetal lung and heart development, we explored the potential implication of this signaling pathway in an experimental model of CHD. Pregnant Sprague-Dawley rats were exposed to either 100 mg nitrofen or olive oil on embryonic day 9.5. On embryonic days 17 and 21, fetuses were delivered by caesarian section, sacrificed, checked for CDH and their lung and heart tissue were harvested for pathobiological evaluation.
Lung and heart weight-to-body weight ratios decreased by 28% and 35% ($P<0.05$) on embryonic day 17 and by 12% and 8% ($P<0.05$) on embryonic day 21. Nitrofen administration resulted in airway septa thickening, together with lower radial alveolar count. The pulmonary expressions of the BMP receptor (BMPR) type 2, BMP4 and BMP7 decreased, while the expression of BMPR1A did not change and the expression of gremlin, a BMP antagonist, increased on embryonic day 17. The pulmonary expression of DNA binding protein 1 (Id1) decreased, together with decreased pro-apoptotic Bax/Bcl2 ratio on embryonic day 21. The myocardial expressions of BMPR2, BMPR1A, BMP7 and SERCA-2A were decreased, while the expressions of gremlin and noggin increased on embryonic day 17. On embryonic day 21, the myocardial expressions of Id1 and SERCA-2A decreased, while gremlin expression increased.
These results suggest that BMP signaling is downregulated in the lungs and the heart at early and late stages of nitrofen-induced CDH.

P3904
TSC1/mTOR pathway promotes hypoxia-induced pulmonary hypertension in mice
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Background: Chronic hypoxia is a key trigger of pulmonary vascular remodeling in pulmonary hypertension (PH). The mammalian target of rapamycin (mTOR) is involved in cell proliferation, which is negatively regulated by Tuberous sclerosis complex 1(*TSC1)*. However, whether TSC1/mTOR pathway is involved in hypoxia-induced PH is still unknown.
Objective: To find whether TSC1/mTOR pathway is involved in PH and provide a target for its therapy.
Methods: Endothelial cell-specific mutation of *TSC1 in mice (*Tek-cre(+)/TSC1$^{fx/+}$ and Tek-cre(-)/TSC1$^{fx/+}$) (provided by prof. Kai-feng Xu) were used. The mice were exposed to either hypoxia(10% O_2) or normoxia (21% O_2), then the right ventricular systolic pressure(RVSP), and index of right ventricular hypertrophy(RVHI) were measured. Histological measurement was used to estimate the distal vascular remodeling. Western blot was used to detect the change of protein expression in mice lungs.
Results: Those two genotypic mice under normoxia showed no differences. After hypoxia, RVSP and RVHI of those two genotypic mice was gradually increased, but Tek-cre(+)/TSC1$^{fx/+}$ mice were higher after 3 weeks (RVSP: 22.79±0.31 vs. 19.95±0.97mmHg, $p<0.05$; 0.32±0.01 vs. 0.25±0.02, $p<0.05$). The small pulmonary arteries of both Tek-cre(+)/TSC1$^{fx/+}$ and Tek-cre(-)/TSC1$^{fx/+}$ showed progressive medial thicken under hypoxia, but the former was more obvious. The expression of phosphorylation of S6 (biomarker of mTOR) gradually increased in lungs of these two genotypic mice exposed to hypoxia in the first 2 weeks, then decreased. And Tek-cre(+)/TSC1$^{fx/+}$ mice showed higher amounts.
Conclusion: TSC1/mTOR pathway can promote hypoxia-induced PH in mice, which provides a novel target for PH therapy.

P3905
Feasibility of eccentric exercise training (ECCt) in monocrotaline (MT) rats: Effects on survival, echocardiographic and hemodynamic parameters
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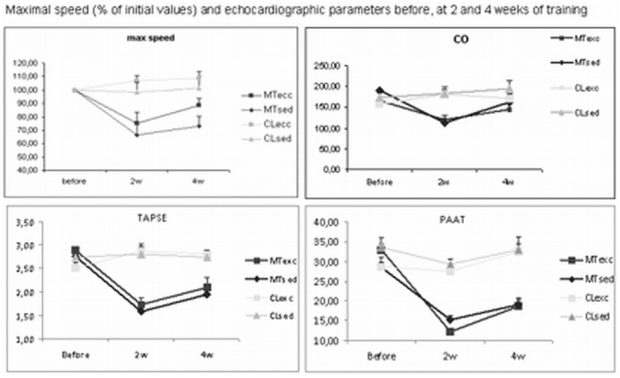
ECCt may be of interest in patients with pulmonary hypertension (PH) because cardiac solicitation is much lower during ECC than during concentric exercise, performed at the same mechanical power.
Aims: We assessed the feasibility, hemodynamic and survival effects of ECCt in MT rats with PH.
Methods: ECCt on treadmill was initiated 2 weeks after MT injection (40mg/kg) (30 minutes at 50% of maximal speed, slope: -15°, 5 days/week for 4 weeks). Trained rats (MTecc, n=13, control ECC (CLecc), n=7) were compared with sedentary rats (MTsed, n=13, CLsed, n=7). Before and after 2 and 4 weeks training, maximal speed measurement and echocardiography were performed. At 4w, right ventricular (RV) catheterisation was performed.
Results: The RV systolic pressure was 40±2 mmHg in MT, and 22±1mmHg in CL rats ($p<0.001$). Exercise was generally well tolerated. In the MTecc and MTsed groups, 3 and 2 rats developed right heart failure and died. Maximal speed significantly increased in trained rats at 4 w ($p<0.001$) [figure]. Echocardiographic parameters were not significantly different in MTsed and MTecc (cardiac output (CO), tricuspid annular plane systolic excursion (TAPSE), pulmonary artery flow acceleration time (PAAT) [figure]. RV systolic pressure was not different in MTecc (36±4mmHg) and in MTsed (42±3mmHg).



Conclusion: In this PH model, ECCt was well tolerated and not detrimental to the hemodynamic condition and survival. Whether ECCt could be an adjuvant therapy in PH deserve to be further investigated.

P3906
Superior in vivo efficacy of macitentan: Comparison to other endothelin receptor antagonists
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Endothelin (ET) receptor antagonists used for the treatment of pulmonary arterial hypertension present different pharmacological profiles depending on their selectivity and affinity for ET receptors. Macitentan (MACI) is a new dual ETA/ETB tissue targeting receptor antagonist designed to achieve a more complete ET receptor blockade. To investigate this property, we designed a study in which rats were given MACI on top of maximally effective doses of either ambrisentan (AMBRI, ETA selective) or bosentan (BOS, dual ETA/ETB).
First, we measured the effects of single doses of the compounds on mean arterial blood pressure (MAP) in conscious Dahl salt-sensitive rats equipped with telemetry, and constructed dose-response curves. Maximal effective doses were 30 mg/kg for MACI and AMBRI and 100 mg/kg for BOS.
Next, we tested the potential for an additive effect of MACI on top of the now defined maximal effective doses of AMBRI and BOS.
MACI 30 mg/kg further decreased MAP by 17 mmHg when given on top of AMBRI 30 mg/kg ($p<0.05$ vs vehicle). In contrast, addition of AMBRI 30 mg/kg on top of AMBRI 30 mg/kg had no additional effect (5 mmHg vs vehicle, p=0.47), confirming use of the maximal effective dose of AMBRI. Conversely, AMBRI 30 mg/kg given on top of MACI 30 mg/kg failed to induce any additional MAP decrease.
In a similar experiment, MACI on top of maximal effective dose of BOS elicited a further MAP decrease of 21 mmHg ($p<0.02$ vs vehicle), whereas addition of BOS had no additional effect.
The add-on effect of macitentan on top of ambrisentan or bosentan confirms that this novel compound is able to achieve a more complete blockade of ET receptors and provides evidence for superior efficacy potential.

P3907

Resveratrol attenuates hypoxic pulmonary vascular remodeling in simulated high altitude-exposed rats: potential role of Hif-1α/NOX4/ROS inhibition

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Objectives: Chronic high altitude hypoxia induces pulmonary vascular remodeling with medial hypertrophy and luminal narrowing leading to the development PAH. Pulmonary oxidative stress has been implicated in hypoxic PAH. This study aimed to investigate the effects of resveratrol, an anti-oxidant polyphenol, on hypoxic pulmonary vascular remodeling in rats.
Methods: Rats were exposed to simulated high altitude of 6000 m in a hyperbaric chamber for 8 h/d, for up to 28 days. Resveratrol (10 mg/kg, ip) was daily administered 0.5 h before hypoxia exposure. Rat primary pulmonary arterial smooth muscle cells (PASMCs) were incubated under hypoxia (2% O_2) in the presence of 10, 25, or 50 μM resveratrol. Pathophysiological changes and signal transduction were examined using histochemistry, fluorescence probing, Western blotting and RT-PCR.
Results: Resveratrol administration significantly reduced hypoxia-induced elevation in mPAP (23.6±2.4 mmHg *vs.* 30.3±1.9 mmHg; $P<0.05$) and medial wall thickness of pulmonary arterioles (16.5±1.8% *vs.* 22.7±2.4%; $P<0.05$) in rats. Resveratrol also decreased pulmonary MDA and H_2O_2 levels as indicators of oxidative stress in hypoxic PAH rats. In vitro studies show that resveratrol dose-dependently inhibited hypoxia-induced rat PASMC proliferation and cellular ROS accumulation. Moreover, resveratrol reduced hypoxia-increased Hif-1α and NOX4 (a ROS contributor) expression both in vitro and in vivo.
Conclusions: Resveratrol attenuates hypoxic pulmonary vascular remodeling in rats exposed to intermittent simulated high altitude, possibly through its inhibition on Hif-1α/NOX4/ROS-generated oxidative stress under hypoxia.

P3908

Applying pharmacogenomics to pulmonary arterial hypertension (PAH): A target-based approach to therapy

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Introduction & aims: Pharmacogenomics, the study of how genetic variations influence the response to drugs, has the desirable objective of tailor-making drugs for each individual genetic makeup. The successful application of this concept in oncology provided the rationale for this study. Like cancer, PAH is a heterogeneous disorder with an unsatisfactory outlook, where responses to drugs often differ in different forms of the disease.
Methods: We examined 2 experimental models of PH: mice with deletion of the Vasoactive Intestinal Peptide gene ($VIP^{-/-}$), and rats injected with monocrotaline (MCT), 2 models with comparable, though not identical, phenotypic features. We analyzed their particular gene alterations, with special reference to genes related to vascular remodeling and inflammation, and compared phenotypic and genotypic responses in each model to treatment with VIP.
Results: $VIP^{-/-}$ mice showed overexpression of genes promoting vascular proliferation and inflammation, with underexpression of anti-proliferative genes. VIP fully corrected all PH features and matching gene expression alterations. MCT rats, however, showed complex gene expression alterations: As in $VIP^{-/-}$ mice, those promoting vascular remodeling and inflammation, and others tending to modulate the PH. Further, VIP treatment failed to correct many of the genotypic abnormalities, and only partially corrected the phenotype.
Conclusions: This preliminary proof-of-concept study demonstrates the importance of genomic information in determining the therapeutic response to a given drug. Full validation of the role of pharmacogenomics in PAH must await comparable studies in patients with different forms of the disease.

P3909

Resolution of venous thrombus is depending on B-lymphocytes

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Purpose: Splenectomy is associated with complex venous thromboembolism such as recurrent deep venous thrombosis, portal vein thrombosis, and chronic thromboembolic pulmonary hypertension (CTEPH). The spleen serves not only as a red blood cell filter but also as immunological organ. The aim of our study was to decipher the population of spleen cells responsible for misguided thrombus resolution after splenectomy.
Methods: We utilized a mouse model of stagnant flow venous thrombosis to characterize thrombus resolution. Splenectomy was performed one month before vena cava ligation. In defined groups, whole spleens, spleens depleted of B-lymphocytes or B-lymphocytes alone were reinfused intraperitoneally. On days 3,7,14 and 28 after vena cava ligation thrombi were harvested for histology.
Results: Thrombi of splenectomized mice were significantly larger than those of controls at all time points (ANOVA, n=8, $p<0.03$). Reinfusion of autologous whole spleen-homogenates reconstituted a normal pattern of thrombus resolution/organisation. Reinfusion of spleen tissue depleted of B-lymphocytes did not affect thrombus resolution. However, reinfusion of autologous splenic B-lymphocytes in previously splenectomized mice normalized thrombus resolution (Fig. 1).

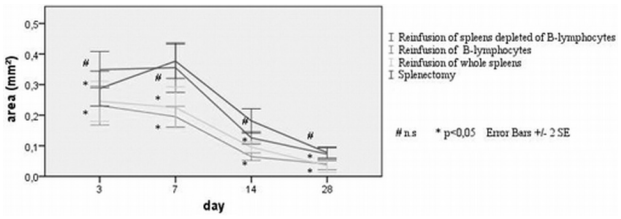

Figure 1

Discussion: Reinfusion of spleen cells restores normal venous thrombus resolution in a mouse model. Our data demonstrate that splenic B-lymphocytes play a key role in thrombus resolution.

P3910

Prevention and partial reversal of monocrotaline-induced pulmonary arterial hypertension (PAH) by hsp90 inhibitors

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We tested the hypothesis that the hsp90 inhibitor, 17-AAG might reverse the hemodynamic alterations associated with PAH. PAH was induced in rats by a single injection of monocrotaline (MCT, 60mg/kg, sc). Four weeks later, a subset of these animals began receiving 17-AAG (1mg/kg 3xweekly, ip) for an additional two weeks. Hemodynamics were monitored weekly by high resolution echocardiography. Right ventricular systolic pressure (RVSP) was also measured invasively at 4 and 6 weeks after MCT. Pulmonary acceleration time (PAT) decreased progressively at 4 and 6 weeks after MCT and reached its nadir at 6 weeks. Similarly, cardiac output (CO) and velocity time integral (VTI) decreased progressively at 4 and 6 weeks post MCT and reached their nadir at 6 weeks. 17-AAG reversed the increase in CO and VTI. At 6 weeks post MCT, both values were statistical similar to those prior to MCT. At 6 weeks post MCT, PAT values in rats treated with 17-AAG between weeks 4 and 6, were higher than those in rats receiving MCT alone for 6 weeks, but similar to those in rats receiving MCT alone for 4 weeks. RVSP and the ratio of the right ventricular weight to either the left ventricular + septum weights (RV/LV+S) or to body weight (RV/BW) increased progressively in MCT-treated rats and reached their maximum at 6 weeks after MCT. At 6 weeks post MCT, RVSP, RV/LV+S and RV/BW values in animals treated with 17-AAG between weeks 4 and 6 were lower than those in rats receiving MCT alone for 6 weeks, but similar to those in rats receiving MCT alone for 4 weeks. These findings suggest that hsp90 inhibitors prevent and partly reverse cardiopulmonary hemodynamic changes associated with MCT-induced PAH.

P3911

Profibrotic cytokine TGF-β1 increases endothelial progenitor cell angiogenic properties

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Background: TGF-β1 is a profibrotic cytokine that plays a major role in vascular biology, and is known to regulate the phenotype and activity of various vascular cell populations. Because most fibrotic diseases, such as Idiopathic Pulmonary Fibrosis (IPF), are associated with vascular remodeling and since endothelial progenitor cells may be involved in this process, we investigated the impact of TGF-β1 modulation of endothelial progenitor cell angiogenic properties.
Patients/methods: TGF-β1 plasma levels were determined in 64 patients with IPF and compared to controls. The effect of TGF-β1 on angiogenesis was studied in vivo in a Matrigel plug model and in vitro on Endothelial Colony Forming Cells (ECFCs). We studied the effects of inhibiting the expression of the three main receptors of TGF-β1 in ECFCs using siRNA.
Results: Total TGF-β1 plasma levels were significantly increased in patients with IPF compared to controls ($P < 0.0001$). TGF-β1 had proangiogenic effects in vivo by increasing hemoglobin content and blood vessels formation in Matrigel-plugs implanted in C57/Bl6 mice and in vitro by enhancing ECFC viability and migration. The effects were abolished by silencing the three main TGF-β1 receptors.
Conclusions: TGF-β1 is pro-angiogenic in vivo and induces ECFC angiogenic properties in vitro, suggesting that TGF-β1 may play a role during vascular remodeling in fibrotic disease states via endothelial progenitor cells.

P3912

Unique receptor dissociation kinetics of the novel endothelin receptor antagonist macitentan

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Association and dissociation rates of G protein-coupled receptor antagonists can influence their in vivo pharmacological activity, such as duration of action, activity in situations of increased agonist concentrations and ultimately clinical efficacy.
Using signaling assays in human pulmonary arterial smooth muscle cells (PASMC), we investigated the endothelin (ET) receptor inhibition kinetics of macitentan, a novel ET receptor antagonist currently in phase 3 clinical trials in pulmonary arterial hypertension, and compared them with the kinetics of bosentan and ambrisentan.
Calcium flux assays showed that macitentan, but not ambrisentan or bosentan, increased in potency (10-fold) upon prolongation of antagonist pre-incubation time from 10 min to 120 min, indicating slow apparent association of macitentan with ET receptors. Macitentan furthermore displayed a slow receptor dissociation rate, as inhibition of ET-1-induced calcium flux persisted for more than 60 min after macitentan wash-out. Conversely, bosentan and ambrisentan did not maintain receptor blockade after washout and displayed a ~15-fold shorter receptor occupancy half life than macitentan. The slow dissociation kinetics rendered macitentan an efficient antagonist of ET-1-induced IP3 synthesis and ET-1-induced sustained calcium flux across the whole range of ET-1 concentrations tested. In contrast, bosentan and ambrisentan did not display antagonism against high ET-1 concentrations in these assays.
In pulmonary arterial smooth muscle cells, macitentan is a slow-offset competitive antagonist and, unlike ambrisentan and bosentan, capable of efficient receptor blockade in functional assays irrespective of the ET-1 concentration.

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Pulmonary gene expression of tenascin-C and fibronectin in chronic obtructive pulmonary disease (COPD)

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Background: COPD is characterized by pulmonary vascular remodeling and is associated with high prevalence of cardiovascular events regardless of other risk factors. Tenascin-C (TNC) and fibronectin (EDA-FN) are extracellular matrix proteins involved in cardiovascular pathology and in lung fibroproliferative diseases. Our principal aim was to elucidate whether this lung gene expression is different between COPD and smokers patients.
Methods: Specimens of lung tissue from two groups, COPD and smokers without COPD, both who required surgery for lung cancer were studied. Clinical and spirometric data were collected. Gene expression was measured by quantitative polymerase chain reaction (Real time PCR) using comparative method (Ct) and RQ (fold change ratio) as a unit of measure.
Results: Ten of the 28 patientes had COPD (FEV_1, 69*m*3% ref.; DLCO, 67±5% ref.) and 18 were only smokers (FEV_1, 92±3% ref.; DLCO, 86±4% ref.). There was no difference in the prevalence of cardiovascular risk factors between groups. The expression of TNC and EDA-FN (mean±SEM) in COPD group was 17,6±6 and 5,1±1 respectively, versus 15,5±5 and 3,9±1 in the control group (p NS). In multivariate analysis, only the active smoking was associated with higher expression of TNC (β=24,588; p=0,002) but not with the COPD condition.
Conclusions: In our study, there were no differences in the gene expression of TNC and EDA-FN in lung tissue of COPD patients compared to smokers without COPD. However, we found an increased expression of TNC in relation to active smoking, so it might have a role in the pathogenesis of COPD, although the mechanisms involved in cardiovascular disease require other specific studies.
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P3914

Effects of a soluble guanylate cyclase stimulator, BAY 41-8543, upon right ventricular function in experimental pulmonary embolism

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Background: Pulmonary embolism (PE) increases pulmonary vascular resistance (PVR) and may cause right ventricular (RV) dysfunction, leading to poor clinical outcome. Our studies show BAY 41-8543 reduces PVR in experimental PE (Watts, J.A. et al., Crit Care Med 2011; 39:2700-2704), but effects upon RV function were previously unknown.
Aims: The studies test if soluble guanylate cyclase stimulator (BAY 41-8543) treatment protects RV function in experimental PE.
Methods: Experimental PE was induced in anesthetized, Sprague-Dawley rats by infusing 25μm polystyrene microspheres in the right jugular vein producing severe PE (2.6 million/100 gm body wt, 5 hrs) or moderate PE (2.0 million/100 gm, 18 hrs). PVR was estimated in-Vivo. Heart function was studied ex-Vivo using Langendorff technique.
Results: Moderate PE produced a significant 3-fold increase in PVR, which was prevented by BAY 41-8543 (50 μg/kg, I.V.) treatment (Control 0.6±0.04; PE 1.9±0.2; PE + BAY 41-8543 0.7±0.04 mmHg/ml/min). Moderate PE caused a significant decrease in RV peak systolic pressure (PSP, Control 39±1 mmHg vs. 19±3 PE), +dP/dt (1192±93 mmHg/sec vs. 444±64) and -dP/dt (-576±60 mmHg/sec vs. 278±40). BAY 41-8543 significantly improved all three indices of RV function (PSP 35±3.5, +dP/dt 1128±100, -dP/dt -568±87). Severe PE caused significant RV dysfunction (PSP 26±2, -dP/dt -369±29) and BAY 41-8543 protected RV function (PSP 34±2, -dP/dt -535±41).
Conclusions: Experimental PE increases PVR and produces RV dysfunction. Treatment of the animals with the soluble guanylate cyclase stimulator, BAY 41-8543, reduces PVR and improves RV function.

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Fluorescence activated cell sorting for simultaneous assessment of nine surface markers of circulating endothelial progenitor cells in pulmonary hypertension

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Background: The role of circulating endothelial progenitor cells (EPCs) in pulmonary hypertension (PH) patients is unknown. In this pilot study we established a nine-colour staining assay for the Fluorescent Activated Cell Sorting (FACS) to characterize the circulating EPCs in PH patients as compared to healthy controls.
Patients and methods: Peripheral and central venous blood was taken from PH patients and healthy controls. Mononuclear blood cells were isolated by means of density gradient centrifugation. The cells were simultaneously stained with fluorescent conjugated antibodies against the cell surface markers c-kit, CXCR2, VEGFR2, CD34, CD14, CD31, CD133, CD16, and CD45. EPCs were defined as CD34+ CD133+ VEGFR2+ cells.
Results: N=10 PH patients (n=4 idiopathic, n=3 chronic thromboembolic, n=3 left heart disease), mean pulmonary artery pressure: 42±14 mmHg, pulmonary vascular resistance: 539±290 dyn·s/cm^5) and n=10 healthy controls were included. No spectral overlap occurred during the nine-colour staining assessment. Circulating EPCs counted from peripheral and central blood revealed no significant differences. All cells were CD45+, suggesting their hematopoietic origin. CD34+ cells were significantly decreased in PH patients as compared to controls (0.3% vs. 1.8% of the mononuclear gate, $p<0.005$). EPCs were significantly lower in PH patients vs. control (2% vs. 44% of CD34+CD133+ cells, $p<0.0001$).
Conclusion: These preliminary results suggest that multi-colour FACS is suitable for EPC quantification and characterization. Further studies are necessary to define distinct circulating EPCs as markers of PH.