The pulmonary vascular effects of PDGF and Imatinib in murine precision cut lung slices

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vorgelegt von

Christian Cranen

Aus Aachen

Berichter: Professor Dr. rer. nat. Christian Martin
Universitätsprofessor Dr. med. Jan Spillner

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µl	*Microliter*
µmol/l	*Micrometer*
µmol/l	*Micromole/l*
5-HT	*Serotonin; 5-hydroxytryptamin*
6MWD	*6-minute walking distance*
A	*Artery*
AEC	*Alveolar epithelial cell*
AP -1	*Activator protein 1*
AVCRL1 = ALK1	*Activin receptor like kinase type 1*
BMP2	*Bone morphogenetic protein 2*
BMPR2	*Bone morphogenetic protein receptor 2*
Ca^{2+}	*Calcium (ionized)*
CaCl	*Calcium chloride*
CaM	*Calmodulin*
CamKII	*Calcium/calmodulin-dependent kinase II*
cAMP	*Cyclic adenosine monophosphate*
CAV1	*Caveolin-1*
cGMP	*Cyclic guanosine monophosphate*
CO_2	*Carbon dioxide*
COPD	*Chronic obstructive lung disease*
COX1	*Cyclooxygenase 1*

CRE	*cAMP response element*
CTEPH	*Chronic thromboembolic pulmonary hypertension*
CX3CR	*CXC Chemokine receptor type 3*
CXCL12	*CXC-Motif Chemokine*
CXCR4	*CXC Chemokine receptor type 4*
DAG	*Diacylglycerol*
DPG	*Diastolic pressure gradient*
ECM	*Extracellular matrix*
EGF	*Epidermal growth factor*
ENG	*Endoglin*
eNOS	*Endothelial NO Synthase*
EPC	*Endothelial progenitor cell*
ERA	*Endothelin Receptor Antagonist*
Erk 1 / 2	*Extracellular signal-regulated kinase 1 / 2*
ET-1	*Endothelin-1*
FC	*Functional Class*
FGF	*Fibroblast growth factor*
FKN	*Fractalkine*
fmol/l	*Femtomole/l = 10^{-15} mole/l*
g	*Gram*
GF	*Growth factor*
GPCR	*G-protein coupled receptor*
Grb2	*Growth factor receptor-bound protein 2*

h	*Hours*
HEPES	*4-(2-hydroxyethyl)-1-peperazineethanesulfonic acid*
HGF	*Hepatocyte growth factor*
HHV	*Human herpes virus*
HIF	*Hypoxia inducible factor*
HIV	*Human immunodeficiency virus*
i(Ca^{2+})	*intracellular concentration of Ca^{2+}*
IL-1	*Interleukin-1*
IL-6	*Interleukin-6*
IP_3	*Inositol-trisphosphate*
JAK -2	*Janus-activated kinase 2*
K^+	*Potassium*
K^+v-channels	*Voltage-gated potassium channels*
KCl	*Potassium chloride*
KCNK3	*K^+ channel NK3*
KSR	*Kinase suppressor of Ras*
l	*liter*
LHD	*Left heart disease*
L-NAME	*NG-nitro-L-arginine methyl ester*
LTCC	*L-type calcium channel*
LV	*Left ventricle*
MAPK	*Mitogen-activated protein kinase*
MCP-1	*Monocyte chemoattractant protein-1*

MCT	*Monocrotaline*
$MgSO_4$	*Magnesium sulfate*
min	*Minutes*
ml	*Millilitres*
MLC	*Myosine light chain*
MLCK	*MLC kinase*
MLCP	*MLC phosphatase*
mmol/l	*Millimole/l = 10^{-3} mole/l*
MMPs	*Matrix metalloproteinases*
mPAP	*Mean pulmonary arterial pressure*
mPAWP	*Mean pulmonary arterial wedge pressure*
mRNA	*Messenger ribonucleic acid*
NaCl	*Sodium chlorid*
NADP	*Nicotinamide adenine dinucleotide phosphate*
NaH_2PO_4	*Monosodium phosphate*
$NaHCO_3$	*Sodium hydrogen carbonate*
nmol/l	*Nanomole/l = 10^{-9} mole/l*
NO	*Nitric oxide*
NYHA	*New York Heart Association*
PA	*Pulmonary artery*
PAEC	*Pulmonary artery endothelial cells*
PAH	*Pulmonary arterial hypertension*
PAI-1	*Plasminogen Activator Inhibitor-1*

PAP	*Pulmonary arterial pressure*
PASMC	*Pulmonary arterial smooth muscle cell*
PGI_2	*Prostacyclin, Prostaglandin I_2*
PCLS	*Precision-cut lung slices*
PDE	*Phosphodiesterase*
PDGF	*Platelet-derived growth factor*
PDGFR	*PDGF receptor*
PH	*Pulmonary hypertension*
PI3K	*Phosphatidylinositol 3-kinase*
PKC	*Protein kinase C*
PKG	*Protein kinase G*
PLC	*Phospholipase C*
pmol/l	*Picomole/l = 10^{-12} mole/l*
PPHN	*Persistent pulmonary hypertension of the newborn*
PTB	*Phosphotyrosine binding domains*
PV	*Pulmonary vein*
PVH	*Pulmonary venous hypertension*
PVP	*Pulmonary venous pressure*
PVR	*Pulmonary vascular resistance*
Raf	*Rapidly growing fibrosarcoma*
ROCK	*Rho kinase*
ROS	*Reactive oxygen species*
s	*Second*

SAP	*Systemic arterial pressure*
SEM	*Standard error of the mean*
sGC	*Soluble guanylate cyclase*
SH2	*Src homology 2 domain*
SMC	*Smooth muscle cell*
SOCS	*Suppressor of cytokine signaling*
SOS	*Son of sevenless*
STAT	*Signal transducer and activator of transcription*
TGF-β	*Transforming growth factor beta*
TKI	*Tyrosine kinase inhibitor*
TNF-α	*Tumor necrosis factor-α*
TPG	*Transpulmonary gradient*
TRPC	*Transient receptor potential channels*
TXA_2	*Thromboxane A_2*
V	*Vein*
VEGF	*Vascular endothelial growth factor*
VIP	*Vasoactive intestinal peptide*
VSMC	*Vascular smooth muscle cell*
WU	*Wood Unit*

1. Introduction

1.1 Pulmonary Hypertension

1.1.1 Epidemiology and Classification

Pulmonary hypertension (PH) is a progressive disease of various etiologies characterized by increased pulmonary vascular resistance (PVR) and elevated right ventricular afterload. Finally, it can result in right ventricular failure which is associated with a poor prognosis (Benza et al. 2010, 2012; D'Alonzo et al. 1991).

PH definition has been currently changed to a mean pulmonary arterial pressure (mPAP) ≥ 20 mmHg at rest measured by right heart catheterization in combination with an increased PVR ≥ 3 Wood Units (Simonneau et al. 2019). Depending on the etiology, PH affects varying parts of the pulmonary vascular bed which results in significantly different pathophysiology, treatments and prognoses (Benza et al. 2012; Gerges et al. 2015; Schmeisser et al. 2013; Simonneau et al. 2019).

The definition of PH comprises various phenotypes of pulmonary vascular disease. After the 6th World Symposium on PH in 2018, the current classification of PH distinguishes five major categories of PH based on etiological and pathophysiological criteria as well as clinical presentation (Fig.1, Simonneau et al. 2019).

Table 1: *Modified classification of Pulmonary Hypertension, 6th World Symposium on PH, Nice, 2018.*

Classification of PH modified by 6th World Symposium on PH

1. Pulmonary arterial Hypertension (PAH)
1.1 Idiopathic PAH
1.2 Heritable PAH *1.2.1 BMPR2* *1.2.2 ALK1, ENG, SMAD9, CAV1, KCNK3* *1.2.3 Unknown*
1.3 Drug- and toxin-induced PAH
1.4 PAH associated with *1.4.1 Connective tissue diseases* *1.4.2 HIV infections* *1.4.3 Portal hypertension* *1.4.4 Congenital heart diseases* *1.4.5 Schistosomiasis*
1.5 PAH long-term responders to calcium channel blockers
1.6 PAH with overt features of venous/capillaries involvement (pulmonary veno-occlusive disease/pulmonary capillary haemangiomatosis)
1.7 Persistent PH of the newborn syndrome
2. PH due to left heart disease
2.1 PH due to heart failure with preserved LVEF
2.2 PH due to heart failure with reduced LVEF

Classification of PH modified by 6th World Symposium on PH

2.3 Valvular heart diseases
2.4 Congenital/acquired cardiovascular conditions leading to post-capillary PH
3. PH due to lung diseases and/or hypoxia
3.1 Obstructive lung diseases
3.2 Restrictive lung disease
3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern
3.4 Hypoxia without lung disease
3.5 Developmental lung disorders
4. PH due to pulmonary artery obstrictions
4.1 Chronic thromboembolic PH
4.2 Other pulmonary artery obstruction
5. PH with unclear/multifactorial mechanisms
5.1 Hematological disorders
5.2 Systemic and metabolical disorders
5.3 Others
5.4 Complex congenital heart disease

Group 1, pulmonary arterial hypertension (PAH), is related to the pulmonary arterial system and also referred to as pre-capillary PH. It is defined by an increased mPAP ≥ 20 mmHg with a normal mean pulmonary arterial wedge pressure (mPAWP) ≤ 15 mmHg and PVR ≥ 3 WU, resulting in an elevated transpulmonary gradient (Breitling et al. 2015; Dadfarmay et al. 2010; Simonneau et al. 2019).

Subgroups 1.1 and 1.2, the idiopathic and hereditary PAH, have both been associated with mutations of bone morphogenetic protein 2 (BMP2), a member of the transforming growth factor beta (TGF-β) family. The survival rate of idiopathic and familial PAH patients after initial diagnosis was found to be 82,9%, 67,1% and 58,2% for 1, 2 and 3 years respectively (Humbert et al. 2010) or 85%, 68%, 57% and 49% for 1, 3, 5 and 7 years respectively (Benza et al. 2012).

The drug- and toxin induced PAH represent subgroup 1.3. Among the substances most likely associated with PAH are the anorexigens Fenfluramine and Dexfenfluramine as well as methamphethamine and the tyrosine kinase inhibitor Dasatinib (Montani et al. 2013; Simonneau et al. 2019).

Subgroup 1.4 comprises PAH connected to connective tissue diseases (1.4.1), PAH associated with HIV infection (1.4.2) and porto-pulmonary hypertension (1.4.3). Subgroup 1.4.4. contains PAH which develops on the basis of increased pulmonary perfusion due to systemic-to-pulmonary shunt within the context of congenital heart disease, leading to increased PAP and pulmonary arterial remodelling. As a consequence, right ventricular hypertrophy occurs, leading to systemic blood pressure in the pulmonary arterial system and to Eisenmenger's syndrome (reversal of the shunt flow). To prevent this lethal development pulmonary arterial banding is performed (Wood 1958). PAH associated with schistosomiasis (1.4.5) is frequent in regions with endemic infection (De Cleva et al. 2003; Lapa et al. 2009).

There is evidence that the PH in patients with a strong acute response to calcium channel inhibitors (Subgroup 1.5) is a distinct entity of PH with a relatively good prognosis when treated with long-term CCI (Simonneau et al. 2019; Sitbon et al. 2005).

Subgroup 1.6, PAH with overt features of venous/capillaries involvement encompasses pulmonary venous occlusive disease and pulmonary capillary hemangiosis, which diseases often occur in combination and are even discussed to be two manifestations of the same condition (Lantouéjoul 2006). Another form of PH with special standing is the persistent pulmonary hypertension of the newborn (PPHN) that was recently made group 1.7 (Simonneau et al. 2019).

Group 2 subsumes the forms of PH resulting from left heart disease, which is likely the most frequent cause of PH (Oudiz 2007). 60-80% of chronic heart failure patients develop pulmonary hypertension, which is associated with a poor prognosis (Schmeisser et al. 2013). The resulting pulmonary venous hypertension (PVH) or post-capillary PH, as opposed to pre-capillary PH of Group 1, primarily affects the pulmonary venous bed, leading to enhanced pulmonary venous pressure (PVP) and PCWP (> 15 mmHg) and eventually also mPAP (≥ 20 mmHg). It was shown, however, that reactive remodelling occurs in both the pulmonary arterial and venous system (Hunt et al. 2013). Post-capillary PH can be divided into isolated post-capillary (Ipc-PH), also known as “passive”, PH and combined pre- and post-capillary (Cpc-PH), also called “reactive” or “out of proportion” PH (Gerges et al. 2015). In Ipc-PH, the mPAP passively increases to overcome the elevated PVP and PCWP but does not rise further. In Cpc-PH, however, reactive remodelling and increased resistance of the precapillary pulmonary vascular bed lead to an out-of-proportion increase of mPAP and therefore a TPG > 12 mmHg (Dadfarmay et al. 2010).The diagnostic tools to distinguish between these two subsets of post-capillary used to be the TPG (> 12 mmHg in Cpc-PH or ≤ 12 mmHg in Ipc-PH) and the pulmonary vascular resistance (> 3 Wood units in Cpc-PH or ≤ 3 Wood units in Ipc-PH). Recently it has been criticised that both TPG and PVR have to be interpreted in the context of dynamic parameters like cardiac output, stroke volume and systolic PAP as well as distension and recruitment of pulmonary vessels, which makes this method susceptible to errors (Schmeisser et al. 2013). To circumvent this problem, the mean diastolic pressure gradient (DPG) is being tested as a less sensitive parameter (Gerges et al. 2015). A distinction between the two subsets of Group 2 PH is necessary because Cpc-PH has a worse prognosis than Ipc-PH and requires a different therapy (see 1.1.3) (Gerges et al. 2015; Schmeisser et al. 2013). To elaborate further on the differences of Ipc-PH and Cpc-PH, while fascinating, would exceed the capacity of this dissertation and bears little relevance to the subject examined here. The interested reader is referred to the works of Schmeisser, Schroetter et al. 2013; Gerges et al. 2015; Dadfarmay et al. 2010; Oudiz 2007, among others.

Important to know, left heart failure constitutes the most common cause of PH (Rosenkranz and Gibbs 2016), however concerning the survival of those patients only

limited data are available. Recent research from Gerges et al. revealed that patients with PH due to left ventricular failure survive 6-10 years from diagnosis (Gerges et al. 2015).

PH can develop from chronic pulmonary diseases and/or chronic hypoxia. This form of PH is classified as Group 3. Studies in several animal models have shown that the resulting changes leading to increased vascular tone and remodelling are complex and affect different parts of the pulmonary arterial system, from large pulmonary arteries (PAs) to non-muscular alveolar wall vessels, in different ways (Stenmark et al. 2006). There is evidence suggesting that the pulmonary venous system is involved in the development of Group 3 PH as well (Andersen et al. 2017).

Acute or chronic pulmonary arterial obstruction can be caused by many different underlying conditions and reduce the functional diameter of the pulmonary vascular system, leading to an increased resistance and PAP (Tapson and Humbert 2006). These forms of PH make up Group 4. Group 5 sums up several different forms of PH with unclear and multifactorial pathogenesis. Hematologic disorders (5.1) that have been shown to be associated with increased prevalence of PAH include mainly chronic myeloproliferative disorders (Peacock 2005; Simonneau et al. 2019). One of the systemic and metabolic disorders (5.2) known to increase the risk of PH is Sarcoidosis by a variety of pathogenetic mechanisms (Bourbonnais and Samavati 2008; Nunes et al. 2006). Subgroup 5.3 consists of several miscellaneous conditions which do not fit into other groups, including end-stage renal disease with long-term hemodialysis (Montani et al. 2013; Simonneau et al. 2019). Finally, PH due to complex congenital heart diseases belong in Subgroup 5.4.

1.1.2 Pathogenesis

The increase of the PVR in PH results from several pathomechanisms, the most prominent of which are remodelling of the small PAs and PVs, increase of the vascular tone, in-situ thrombosis and inflammation. The pathophysiology is likely to vary for different types of PH and has been investigated most extensively for precapillary PH (Humbert et al. 2004; Montani et al. 2013).

1.1.2.1 Vascular tone

Pulmonary blood vessels are built with the characteristic structure of intima, media and adventitia layer like most blood vessels, although the particular pattern of these layers varies depending on the localisation in the pulmonary vascular bed (Stenmark et al. 2006). The media layer is made up mostly of smooth muscle cells (SMC) which contract or relax and thereby determine the vascular tone. In PH, this tone is inadequately high due to increased contraction of the arterial and venous SMC, finally leading to increased PVR. The contraction of SMC works via crossbridge cycling of myosin with actin filaments, promoted by phosphorylation of the myosin light chain by myosin light chain kinase (MLCK) and attenuated by dephosphorylation of the myosin light chain by myosin light chain phosphatase (MLCP) (Stenmark et al. 2006). The activity of both enzymes is regulated by the level of intracellular Ca^{2+} and by the Ca^{2+} sensitivity which describes the effect a given cytosolic Ca^{2+} level has on SMC contraction. The regulation is complex and influenced by many factors both extra- and intracellular, many of which contribute to the increased PVR in PH (Stenmark et al. 2006). The most important ones of these factors and their relevance in PH will be briefly introduced now.

Prostacyclin (prostaglandin I_2, PGI_2), is produced in a wide range of tissues, among them endothelial cells (EC), vascular SMCs and nonvascular SMCs (Majed and Khalil 2012). PGI_2 binds to the prostacyclin receptor (IP) and affects SMCs in a vasorelaxant as well as antiproliferative way. Activation of the G_s-coupled receptor causes an increase of intracellular cAMP/PKA which inhibits MLCK, activates MLCP and opens potassium channels, leading to a successive hyperpolarisation and a reduced opening of voltage gated Calcium channels (Majed and Khalil 2012). Finally, intracellular Calcium levels decrease, leading to reduced cell contractility and proliferation (Majed and Khalil 2012). In addition, PGI_2 inhibits platelet aggregation (Majed and Khalil 2012). In PAH patients the pulmonary expression of PGI_2-synthase is lower than in controls (Christman et al. 1992; Tuder et al. 1999). PGI_2 analogues, such as Iloprost, are established drugs in PAH specific therapy (Barst et al. 2009; Montani et al. 2014).

Endothelial nitric oxide (NO) is produced in ECs by endothelial NO synthase (eNOS). It promotes the synthesis of cyclic guanosine monophosphate (cGMP) which activates protein kinase G (PKG), finally increasing the activity of the myosine light chain phosphatase (MLCP) which itself reduces the calcium sensitivity of SMCs (Lyle et al. 2017). In addition, like PKA, PKG activates potassium channels, leading to hyperpolarisation and reduced Ca^{2+} -influx via voltage gated Ca^{2+} channels (Archer et al. 1994; Lyle et al. 2017). The expression of eNOS is low in lungs of PAH patients (Giaid and Saleh 1995) compared to healthy controls. Inhibition of cGMP degradation by PDE-5-inhibitors like Sildenafil and Tadalafil has long been established in the specific therapy of PAH (Barst et al. 2009; Montani et al. 2014).

Endothelin-1 (ET-1) is expressed by ECs and binds to the G-protein coupled receptors ET_A and ET_B. Binding of ET-1 to ET_A, which is mainly expressed in VSMCs promotes vasoconstriction via $G\alpha_q$ downstream signalling. This includes phospholipase C (PLC) β and inositol triphosphate (IP_3)-mediated elevation of intracellular Ca^{2+} level, as well as increased Ca^{2+} sensitivity which is promoted by protein kinase C (PKC) and rho kinase (ROCK) (Barman 2007; Bouallegue, Daou, and Srivastava 2007; Jeffery and Morrell 2002). Further, ET_A promotes VSMC proliferation and adhesion by activation of phosphoinositide-3-kinase (PI3K)/PKB/Akt and the MAPK pathway (Bouallegue 2007). Different subtypes of ET_B increase the vascular tone in SMCs (ET_B2) and increase the production and release of NO and PGI2 in ECs with a vasodilating and antiapoptotic effect via ET_B1 (Pierce et al. 2002). Both receptors are usually expressed in resistance arteries while in more central and thus larger PAs only ET_A mediates the ET-1 response (Humbert et al. 2004). Both receptor types are expressed in intima and media of normoxic rat pulmonary veins and show increased expression under chronic hypoxia (Takahashi et al. 2001). Further, in animal models of PAH, the levels of ET-1 are significantly higher than in healthy control animals (Giaid et al. 1993). Endothelin receptor antagonists (ERAs) are currently in use in specific PAH therapy (Barst et al. 2009; Montani et al. 2014).

Voltage activated potassium channels (K^+_v-channels) stabilize the membrane potential in resting cells. Inhibition or downregulation of these channels promotes the depolarization of the cell membrane. Consequently voltage activated L-type Ca^{2+}-

channels open and intracellular Ca^{2+} increases, leading to SMC contraction (Montani et al. 2013). Downregulation of the α-subunit of $K_v1.1$, $K_v1.5$, $K_v1.6$, $K_v2.1$ and $K_v4.3$ was found in rat PASMCs during chronic hypoxia, leading to dysfunctional K_v-channels (J. Wang et al. 2005). Additionally, impaired function of K^+v-channels has been reported in PASMCs of PAH patients (J. X. Yuan et al. 1998; X. J. Yuan et al. 1998). Moreover, certain anorexigens known to cause PAH are direct inhibitors of K^+v-channels (Weir et al. 1996). These findings suggest an essential role of K_v-channels in the pathophysiology of PH.

Nonselective cation channels of the transient receptor potential channels (TRPC) family are activated either via depletion of intracellular Ca^{2+} storage (e.g. TRPC1) or receptor-operated (e.g. TRPC3 and TRPC6, via DAG) (Lin et al. 2004). They elevate the cytosolic Ca^{2+} level by promoting Ca^{2+} influx from extracellular or intracellular storages (Lin et al. 2004). In normal rat PASMCs, TRPC1, TRPC3 and TRPC6 are expressed, and in chronic hypoxia the expression of TRPC1 and TRPC6 is upregulated (Lin et al. 2004). TRPC1 and TRPC6 have been shown to be essential to hypoxic pulmonary vasoconstriction in mice (Weissmann et al. 2006). TRPC3 and TRPC6 are upregulated in PAH patients, and inhibition of TRPC6 expression attenuates proliferation of SMC (Yu et al. 2004). Continued influx, both from extracellular and from intracellular storages, is likely to have a mitogenic effect mediated by Calmodulin/Ca^{2+} (Burg et al. 2008). Thus, TRPC channels are likely involved in the disbalance of the cytosolic Ca^{2+} level of PASMCs in PH as means of Ca^{2+} entry alternative to LTCCs.

Serotonin (5-Hydroxytryptamine, 5-HT) is generated in the gastrointestinal tract in enterochromaffin cells and ECs though most 5-HT is stored in platelets. In PAH patients, however, the platelet 5-HT levels are low and the plasma levels elevated (Herve et al. 1995). In PASMCs, 5-HT binds to the G_i-coupled 5-HT-1B receptor and the G_q-coupled 5-HT-2B receptor, as well as to the 5-HTT transporter that internalizes 5-HT into the cell (Stenmark et al. 2006). The 5-HT-1BR has long been associated with hypoxic pulmonary vasoconstriction (Morecroft et al. 1999) and was shown to be crucial to hypoxia-induced PAH (Keegan et al. 2001). Binding to 5-HT-1B, serotonin promotes vasoconstriction via phospholipase C (PLC) β and inositol triphosphate (IP_3)-mediated elevation of intracellular Ca^{2+} level and Ca^{2+} sensitivity (Homma et al. 2007). There is further evidence that the interaction between 5-HT-1BR and 5-HT-2BR promotes the development of PH

(Morecroft et al. 2005). 5-HT plays an essential role in the pathogenesis of PH in humans and in the animal model (MacLean et al. 2000).

Thromboxane A_2 (TXA_2) is a platelet-derived prostaglandin derivative that promotes pulmonary vasoconstriction, thrombogenesis and proliferation. The TXA_2 receptor is primarily coupled to $G_{\alpha q}$ which stimulates IP3/DAG, and to $G_{\alpha 12/13}$ which stimulates Rho-kinase (ROCK)/PKC. Both pathways lead to the increase of intracellular Ca^{2+} and Ca^{2+} sensitivity in rat aortas (Dorn et al. 1992; Nakahata et al. 2000). The TXA_2 receptor also activates a G_i-protein and thereby reduces intracellular cAMP level and PKA activity, again resulting in increased Ca^{2+} sensitivity. Further, it promotes the release of reactive oxygen species (ROS), leading to the inhibition of K^+_v-channels and to SMC depolarization and contraction (Cogolludo et al. 2003). TXA_2 levels have been found to be elevated in PAH patients compared to healthy subjects (Christman et al. 1992).

RhoA is a small GTPase that is activated by interaction of G-protein coupled receptors ($G_{\alpha 12/13}$) with their ligands (e.g. ET-1, 5-HT and TXA_2, see above), but also by acute and chronic hypoxia (Lyle et al. 2017; MacKay et al. 2017). Active RhoA activates ROCK which can also be activated by an elevation of the cytosolic Ca^{2+} level (Lyle et al. 2017). ROCK mediates PASMC contraction by increasing Ca^{2+} sensitivity and there is evidence that it is involved in PDGF-BB induced VSMC proliferation (Loirand et al. 2006). Specific inhibition of ROCK with fasudil causes pulmonary vasorelaxation in intact rats and reverses acute pulmonary hypertension in rats injected with L-NAME or the thromboxane receptor agonist U-46619 as well as hypoxic rats (Badejo Jr. et al. 2008; Dhaliwal et al. 2007). PH in rats induced by either chronic hypoxia or monocrotaline treatment is also attenuated by the ROCK inhibitors fasudil or Y-27632 (Jasinska-Stroschein et al. 2014; Nagaoka et al. 2004). Clinical trials are presently being conducted to assess whether ROCK inhibitors are going to be a potent and safe new drug in the therapy of PAH in the future (Zhang and Wu 2017).

In addition to the abovementioned mediators, there has been increasing data emphasizing the role of platelet derived growth factor (PDGF) in the regulation of the pulmonary vascular tone which will be more extensively discussed in part 2.2 of this introduction.

1.1.2.2 Remodelling

In PH, all vessel layers, i.e. the endothelium, media and adventitia are affected. Hence, ECs, SMCs, fibroblasts, immune cells and platelets are involved with the pathological changes known as remodelling. Of all types of PH, the most extensive research has been done on pre-capillary PH, accordingly pulmonary arterial remodelling has been much more in the focus of attention than pulmonary venous remodelling. Thus, most of the structural and molecular changes introduced below relate to pre-capillary PH and the pulmonary arterial system. However, there is evidence that in several forms of PH a remodelling of the pulmonary venous system occurs that is the subject of ongoing research (Andersen et al. 2017).

In hypoxia-induced PH, the morphological changes vary depending on the species and on the location within the pulmonary vascular bed (Stenmark et al. 2006), however, a thickening of some or all layers of the vascular wall was commonly observed. In the adventitia, the increase in thickness is attributed to an accumulation of fibroblasts and myofibroblasts as well as extracellular matrix (ECM) (Stenmark et al. 2011). In addition, an increased density of vasa vasorum in larger pulmonary arteries has also been described (Davie et al. 2004). Media thickening is a complex process: it is likely promoted by hypertrophy and hyperplasia of different SMC subtypes within the media. Further, fibroblasts, myofibroblasts and progenitor cells migrate from the adventitia and the blood stream, differentiate, proliferate and increase deposition of ECM proteins (Stenmark et al. 2006, 2011). The intima can be invaded by mesenchymal cells from the blood stream or the outer layers (Stenmark et al. 2006) and, in some types of PH, forms obstructive plexiform lesions of the small vessels (Humbert et al. 2004).

There is evidence that pulmonary venous remodelling occurs in both post-capillary PH and idiopathic PAH and that it comprises an arterialization (i.e. smooth muscle cell hypertrophy), a splitting of the elastic layer and intimal fibroelastic thickening as well as increased cellularity (Andersen et al. 2017).

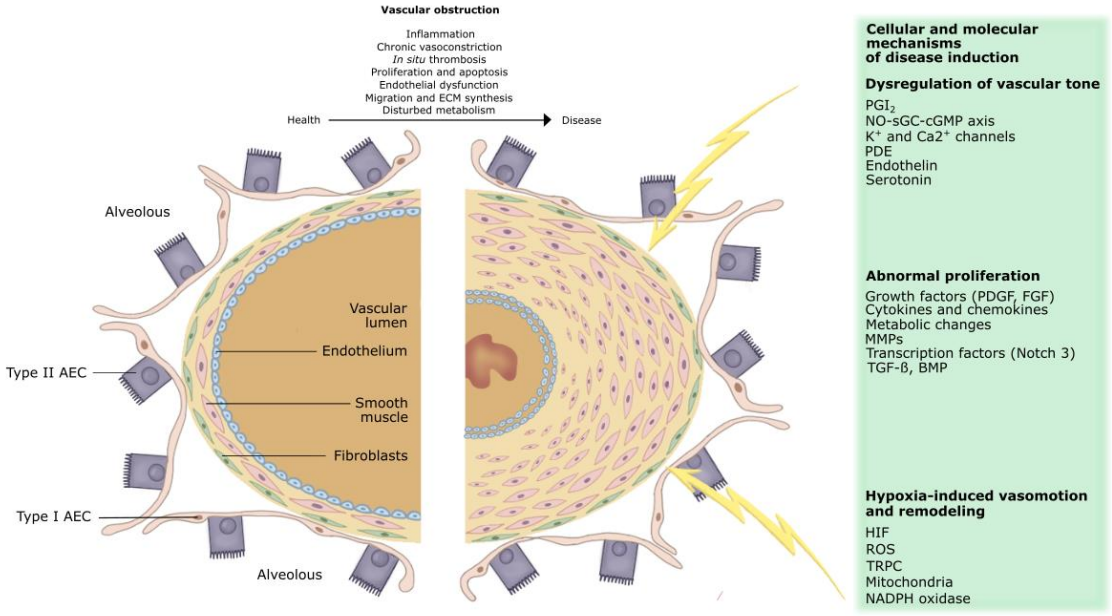

Figure 1: *Vascular remodelling in pulmonary arterial hypertension. Putative therapeutic targets are indicated. Abbreviations: see index.*

Figure adopted with modifications from Schermuly et al. 2011

The expression of several growth factors and their receptors is increased in PH, which stimulates cell proliferation and migration, but also leads to a reduction of apoptosis. Finally, these processes result in the thickening of the vessel wall (Fig.1). Some of the factors involved in this process will be introduced in the following. Beyond that, most of the factors responsible for increased vascular tone, such as ET-1, TXA_2 and 5-HT, also promote proliferation and cell survival and therefore remodelling (see 1.2.1).

Several growth factors are known to play a role in PH pathogenesis. Among those, the TGF-β superfamily, particularly bone morphogenetic protein 2 (BMPR2) (Deng et al. 2000**,** Thomson et al. 2000), stands out because of its high correlation with hereditary and idiopathic PAH.

Platelet derived growth factor (PDGF) has been shown to play an important role in PH (Schermuly et al. 2011), which will be introduced in greater detail in 1.2.

Vascular endothelial growth factor (VEGF) is associated with PAH in a complex and seemingly contradictory manner. Several studies suggest that it is increased in PAH (Geiger et al. 2000; Tuder et al. 2001) and promotes the vascular remodelling (Klein et al. 2008; Moreno-Vinasco et al. 2008). However, other studies found evidence for an attenuating effect of VEGF on PAH (Campbell et al. 2001; Partovian 1998, Farkas 2009). Further research in this field is necessary to illuminate the role of VEGF in PAH.

Several other growth factors might also be involved in the pathogenesis of PAH, e.g. the endothelial growth factor (EGF) family and the hepatocyte growth factor (HGF) (Schermuly et al. 2011).

Further, the hypoxia inducible factor-1 (HIF-1) which regulates the gene expression of some hypoxia-associated cell responses, e.g. survival, angiogenesis and cellular metabolism (Semenza 2001)**,** has been shown to be overexpressed in plexiform lesions in PAH (Tuder et al. 2001). This circumstance is of import, as in mice heterozygote for HIF-1α expression, hypoxia-induced pulmonary vascular remodelling is attenuated compared to homozygote specimen (Semenza 2005).

In addition, the transcription factor Notch3 and its target gene HES5 are discussed to promote PAH due to their overexpression in the PASMC of PAH patients and animal PH models. Conversely, inhibition of Notch receptor expression attenuates the development of PAH in chronic hypoxic mice. Notch3 stimulates SMC proliferation and the expression of PDGFR-β. (Schermuly et al. 2011)

1.1.2.3 Inflammation

Various findings emphasize the involvement of inflammatory elements in the remodelling of pulmonary vessels. In addition to the changes concerning the vascular tone, proliferation, cell survival and migration, the pulmonary ECs release chemokines attracting immune cells and express CXCL12/CXCR4. Those chemokines promote the homing of circulating progenitor cells which differentiate into mesenchymal cells

(Gambaryan et al. 2012; Montani et al. 2013). The endothelium displays disorganized proliferation (Schermuly et al. 2011) and forms characteristic complex plexiform lesions consisting of monoclonal (S. D. Lee et al. 1998) and possibly apoptosis-resistant (Masri et al. 2007) pulmonary ECs, myofibroblasts with extracellular matrix and immune cells such as T- and B-lymphocytes, macrophages, dendritic cells as well as mastocytes (Nicolls et al. 2005; Perros et al. 2007). The exact pathogenesis of these plexiform lesions is incompletely understood. In PAH patients, reduced regulatory T-cell activity may contribute to the fact that pulmonary vessels develop a disposition to autoimmune reactions triggered by stimuli such as pulmonary infection and inflammation (Montani et al. 2013). In PAH patients, the plasma levels of circulating Interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) are elevated compared to control subjects (Humbert et al. 1995; Itoh et al. 2006). Further, the chemokine fractalkine (FKN) and its receptor CX3CR which mediate the endothelial adhesion and tissue immigration of immune cells are overexpressed in circulating T-cells and pulmonary vascular lesions of PAH patients (Balabanian et al. 2002). Interestingly, some infectious agents could be identified to be associated with PAH, among them latent viral infections of HIV and several types of HHV (Hamamdzic 2002). HHV-8 in particular has been found to be latent in a number of PAH patients (Cool et al. 2003). In addition, 20% of long-term Schistosomiasis patients develop PAH (Graham et al. 2010; Simonneau et al. 2013).

1.1.2.4 In-situ-thrombosis

An abnormal platelet function in combination with endothelial dysfunction has thrombogenic effects that can be detected in PAH patients by increased serum levels of fibrinogen A and B, D-Dimers, von-Willebrand-factor and Plasminogen Activator Inhibitor-1 (PAI-1). In this prothrombotic environment, physical factors like shear-stress and endothelial injury promote a continuous in-situ-thrombosis. Platelet dysfunction also affects remodelling and vascular tone due to the various mediators released by platelets (PDGF, VEGF, 5HT, TGFβ, TXA_2) (Herve et al. 1995; Montani et al. 2013).

1.1.2.5 Current therapies

The therapy of PH is variable due to its many different forms. The specific targeted treatment of PH is most widely researched for PAH, or pre-capillary PH. In secondary forms of PH, such as PH due to left heart failure or to lung diseases/hypoxia, there is little conclusive data as of now concerning PH therapy beyond the treatment of the underlying disease (Park et al. 2013; Rosenkranz et al. 2016). Therapy of PAH depends on the PAH functional class (FC) of the respective patient, according to the schema created by the WHO based on the NYHA classification of cardiac insufficiency. The classes correlate to different levels of physical activity at which the PAH becomes symptomatic. Specific PAH drug therapy currently targets three pathways: the PGI_2 pathway (via prostacyclin analoga, e.g. iloprost, epoprostenol and treprostinil as well as the IP receptor agonist selexipag), the endothelin-1 pathway (via endothelin receptor antagonists, e.g. bosentan, ambrisentan and macicentan) and the NO pathway (via PDE-5-inhibitors, e.g. sildenafil and tadalafil as well as the novel soluble guanylate cyclase stimulant riociguat) (Tsai et al. 2016). Furthermore, several new possible drug concepts for targeted PH therapy are being investigated (Aschermann and Jansa 2014; Montani et al. 2014; Sommer et al. 2020).

Activation of the IP receptor by PGI_2, prostacyclin analoga or the receptor agonist selexipag promotes intracellular cAMP production and PKA activity. This causes direct inhibition of MLCK and stimulation of MLCP and the opening of potassium channels in SMCs (see 1.1.2.1), thereby leading to hyperpolarisation and reduced open voltage activated Ca^{2+} channels (Majed and Khalil 2012). The decrease in cytosolic Ca^{2+} level has relaxing, antiproliferative and antimitogenic effects (Majed and Khalil 2012). Further, PGI_2 modulates immune responses and inhibits platelet aggregation (Majed and Khalil 2012). Epoprostenol has a short half-life and needs to be continuously administered.

Therefore, but also due to various side effects it is not widely used anymore (Montani et al. 2014; O'Connell et al. 2012). Recently, an oral form of treprostinil has been approved for PAH therapy (Tsai et al. 2016). Iloprost, which is applicable by inhalation was shown to improve the 6 minute walking distance (6MWD) and the Functional Class (FC) of PAH patients after three months of treatment (Olschewski et al. 2002). Due to the local application of this form of iloprost, systemic side effects are reduced, but not completely prevented. In general, the side effects of all prostanoids comprise headache, flushing and gastrointestinal symptoms (Montani et al. 2014). The new drug selexipag has been shown to significantly reduce mortality in PAH patients both as single treatment and in combination with a continued therapy with endothelin receptor antagonists or PDE-5 inhibitors or both (Sitbon et al. 2015).

Phosphodiesterase type 5 degrades cGMP and therefore also has an inhibitory effect on the activity of protein kinase G (PKG) which is activated by cGMP (see 1.1.2.1). Thus, PDE-5 inhibition promotes cGMP-dependent vasorelaxation, anti-remodelling, pro-apoptotic effects in SMC as well as inhibition of platelet aggregation (Archer and Michelakis 2009; O'Connell et al. 2012). The PDE-5 inhibitors Sildenafil and Tadalafil were both shown to improve exercise capacity, whereas Sildenafil also improved the FC and Tadalafil the time to clinical worsening (Barst et al. 2009; Montani et al. 2014).

Riociguat is a relatively new drug which promotes cGMP synthesis by soluble guanylate cyclase (sGC), both by NO independent stimulation and by making sGC more sensitive for NO (Tsai et al. 2016). In recent clinical trials it improved the exercise capacity as well as several secondary endpoints, e.g. vascular resistance, WHO functional class and time to clinical worsening in patients with PAH and CTEPH (Ghofrani et al. 2013; Rubin et al. 2015).

In PAH patients, ET-1 plasma concentration and ET-1 receptor expression are increased. ET-1 is known to cause vasoconstriction and proliferation of PASMC via activation of the ET-1 receptor A (O'Connell et al. 2012). ET-1 receptor antagonists (ERAs) are orally active drugs. The unselective ERA bosentan inhibits both ET-1 receptors and has been demonstrated to improve 6MWD, hemodynamics, FC and time to clinical worsening in several randomized controlled studies (Montani et al. 2014). Ambrisentan, a specific ET_A

antagonist, was observed to improve symptoms, hemodynamics and time to clinical worsening in several types of PAH (Montani et al. 2014). The most recent ERA, Macicentan, was shown to positively influence the FC, time to clinical worsening and the 6MWD while having relatively mild side effects and drug interactions (Said 2014). ERAs have been shown to cause an elevation of the liver enzymes (Barst et al. 2009). Sitaxsentan is not in use anymore after potentially drug-induced fatal hepatotoxicity (Said 2014).

Recently it has been shown that combination therapy with two different drug classes is superior compared to monotherapy. Thus, the addition of sildenafil to i.v. epoprosterol was observed in a post-hoc analysis to improve survival in patients with severe PAH (Simonneau et al. 2008). Furthermore, an oral dual therapy of treatment-naïve PAH patients with ambrisentan and tadalafil led to a significantly longer time to clinical failure and improved 6MWD compared to a pooled group of patients receiving monotherapy with either drug (Tsai et al. 2016). This suggests that dual therapy might be a promising concept not only in patients with insufficient response to monotherapy but also as initial therapy. However, further studies will be required to ascertain which combinations are the most effective and to investigate interactions between the combined PAH specific drugs (Barst et al. 2009; Montani et al. 2014).

Non-specific drugs recommended in PAH therapy include L-type calcium channel (LTCC)-inhibitors Nifedipin and Diltiazem which are recommended for PAH patients that show a defined „positive acute vasoreactive response“ (drop of PAP and PVR) after inhalation of NO (Sitbon et al. 2005). While an improved prognosis could be shown for these cases (Rich et al. 1992), only half of the patients profiting from this treatment also had a long-term beneficial effect. In patients without “positive acute vasoreactive response”, LTCC-inhibitors are contraindicated due to the danger of decreased cardiac output (Sitbon et al. 2005).

Diuretics relieve the fluid overload and the ensuing damage to liver and gastrointestinal organs resulting from right heart failure. Digoxin increases cardiac output and has antiarrhythmic effects, although its beneficial effect in PAH is not proven (Montani et al.

2014). Oral anticoagulation could be shown to increase three year survival in idiopathic PAH (Olsson et al. 2014).

General measures are important to reduce the risk of acute decompensation in patients of all FCs. There is no evidence-based recommendation as yet concerning an adequate degree of activity but if possible moderate activity should be maintained according to the individual exercise capacity and the symptoms. Female PAH patients are recommended to use contraception because in this group pregnancy leads to a mortality rate of 30%-50%. Mechanical or surgical contraception is preferable to pharmacological methods due to the increased risk of thrombosis of the latter. Some pharmacological drugs such as beta-blockers are contraindicated as well because they restrict the cardiac output, which is often hardly tolerated in PAH patients. In case of necessary surgery, the increased risk of anaesthesia and surgery-related hemodynamic or thromboembolic events must be taken into account (Montani et al. 2014).

Lung transplantation and cardiopulmonary transplantation are a therapeutic option in end-stage PAH. It is an extensive surgery, however, and the five-year survival rate is approximately 50% (Goldberg et al. 2017; Montani et al. 2014). Compared to a five-year survival rate of 57% under specific drug therapy (Benza et al. 2012), this can only be seen as a last resort.

While current therapeutic approaches to PAH have been proven to be effective, we are still far from a sufficient therapy. Novel concepts are currently under investigation.

In PH due to left heart failure (group 2 PH), treatment of the underlying heart failure by the established drug therapy concepts (e.g. ACE inhibitors, beta-blockers, I_f-channel inhibitors, diuretics) and interventional or surgical therapy, particular in mitral valve disease, had beneficial effects on PH as well (Guazzi and Borlaug 2012). However, PH remains associated with a poor prognosis in left heart failure patients and an effective treatment of the pulmonary vascular component of the disease seems desirable (Ghio et al. 2001). Of the established targeted PH drugs introduced above, only sildenafil, a PDE-5 inhibitor, has shown beneficial results (e.g. improved exercise capacity and quality of life) in clinical trials in group 2 PH patients (Guazzi and Borlaug 2012). Nevertheless, the long-term benefit of sildenafil in PH due to LHD is still waiting to be proven. Long

term i.v. treatment with epoprostenol, a PGI_2 analogon, or bosentan, an ERA, led to premature termination of the respective trials due to adverse effects and worsening of heart failure (Califf et al. 1997; Kaira et al. 2002; Kaluski et al. 2008). One possible risk of pulmonary vasodilating drugs is an increase in preload resulting in overload of the diseased left heard, lung edema or systemic hypotension (Breitling et al. 2015). However, group 2 PH is a complex disease, and the safety and efficacy of the traditional PH drugs will have to be examined for homogenous patient populations regarding the etiology of left heart failure (e.g. preserved vs. reduced ejection fraction, heart valve disease etc.) and the subcategory of PH, meaning isolated postcapillary PH (IpcPH) or combined postcapillary PH (CpcPH) (see 1.1.1) to obtain useful data. It is plausible that the traditional targeted PH drug therapy strategy might be more efficient in group 2 PH with a precapillary component (Gerges et al. 2015). Currently, treatment of PH due to left heart disease with targeted PAH drugs is not recommended in the European guidelines (McDonagh et al. 2021; Schmeisser et al. 2013).

1.2 PDGF

1.2.1 PDGF and PDGF receptors

The PDGF family is one of the longest-known growth factor families, having been discovered in the mid-70s (Kohler and Lipton 1974; Ross et al. 1974). PDGF is expressed by both mammals and invertebrates and its physiological role is most important during the development period (Andrae et al. 2008). Structurally, PDGF is a dimer of two polypeptide chains. To date, four types of chains are known: PDGF-A, B, C and D, encoded by nine different genes in mammals (Andrae et al. 2008). Each of those chain types is able to form homodimers to bind to and activate their receptors, and the A- and B-chain can form a functioning heterodimer, PDGF-AB, which has only been found in human platelets yet (Andrae et al. 2008). However, the physiological function of PDGF-AB is not yet fully understood (Andrae et al. 2008). The expression of PDGF and its receptors, PDGFR-α and PDGFR-β, is regulated temporally and locally and exerts its physiological effects on distinct cell groups. While PDGF expression patterns are complex and dynamic, it has generally been observed that PDGF-A and -C are mainly

expressed by epithelium cells, muscle and neuronal progenitor cells, PDGF-B in vascular endothelial cells, neurons and megakaryocytes and PDGF-D in SMCs and fibroblasts. Both receptor types are expressed in mesenchymal cells, however the strongest expression of PDGFR-α occurs in lung, skin and intestine while PDGFR-β is found mainly in vascular SMC (Andrae et al. 2008). The PDGF receptors are transmembrane receptor tyrosine kinases (RTK) that are activated by binding of a PDGF-dimer to its specific extracellular ligand-binding domain, resulting in dimerization and subsequent autophosphorylation and autoactivation of the intracellular tyrosine kinase domains. According to present knowledge the PDGFR-α binds the polypeptide chains PDGF-A, -B and -C, while PDGFR-β is specific for PDGF-B and probably -D (Bergsten et al. 2001; Heldin et al. 1998; Li et al. 2000). However, only a few of the possible ligand-receptor interactions have been shown to be relevant in vivo (Fig. 2, Andrae, Gallini, and Betsholtz 2008).

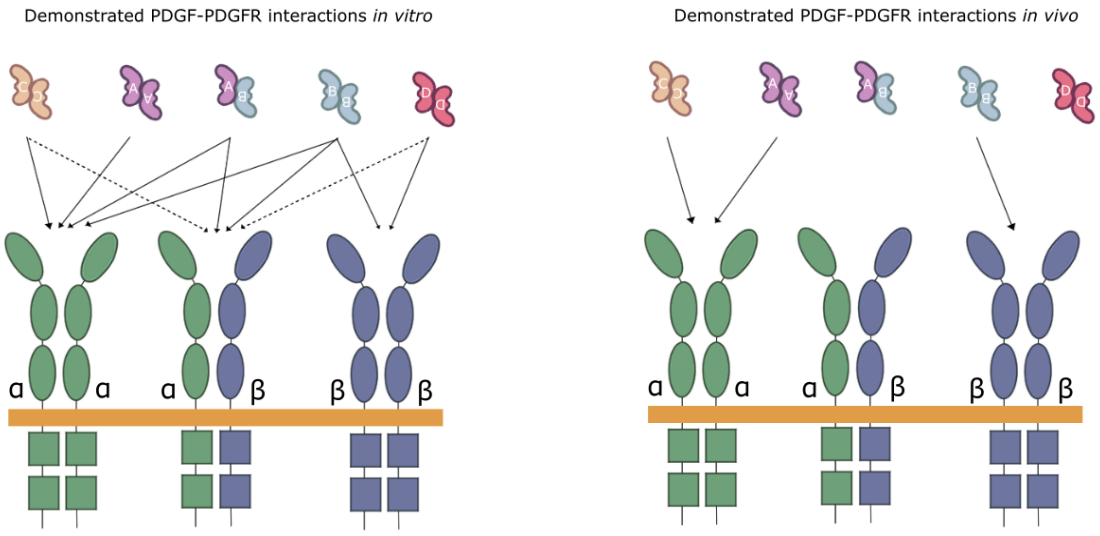

***Figure 2**: PDGF-PDGFR interactions. Each chain of the PDGF dimer interacts with one receptor subunit. The active receptor configuration is therefore determined by the ligand dimer configuration. The left panel shows the interactions that have been demonstrated in cell culture. Hatched arrows indicate weak interactions or conflicting results. The right panel shows interactions proven to be of importance in vivo during mammalian development. Note that PDGF-D has not yet been investigated in this regard.*

Figure adopted with modifications from Andrae et al. 2009

The autophosphorylation of the intracellular tyrosine kinase domain enhances the kinase activity and enables substrates to bind to specific docking sites. These sites display molecular structures, among others phosphorylated tyrosines that are recognized by Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains, proline-rich regions recognized by SH3 domains and membrane phospholipides recognized by Pleckstrin homology (PH) domains, all of which allow the receptor to interact with its substrates (Heldin et al. 1998). These go on to activate the various signalling pathways of the PDGF receptors (Figure 3): several members of the mitogen activated protein kinase (MAPK)-family are activated that promote cell growth, migration and differentiation by regulating transcription. Phosphoinositol 3 kinases (PI3K) activates several effector pathways including protein kinase B (PKB)/Akt, PKC and the rho protein cascade, stimulating cell growth, migration and actin reorganization and inhibiting apoptosis. Phospholipase C (PLC)-γ is activated by PDGF and cleaves membrane bound phosphatidylinositol-4,5 bisphosphate into diacylglycerol (DAG) and inositoltriphosphate (IP_3), causing an increase of intracellular Ca^{2+} and thereby activating PKC. On the cellular level PDGFR-signalling results in enhanced proliferation and migration. PDGF receptors interact further with other protein kinases and integrins, most of which promote proliferation, cell survival and migration. Some of these cellular effects are fast, others slow (Andrae et al. 2008)**.** Further research is required to illuminate the specific differences between signalling of PDGFR-α and PDGFR-β, as well as their role in dependence to the different tissues and developmental phases.

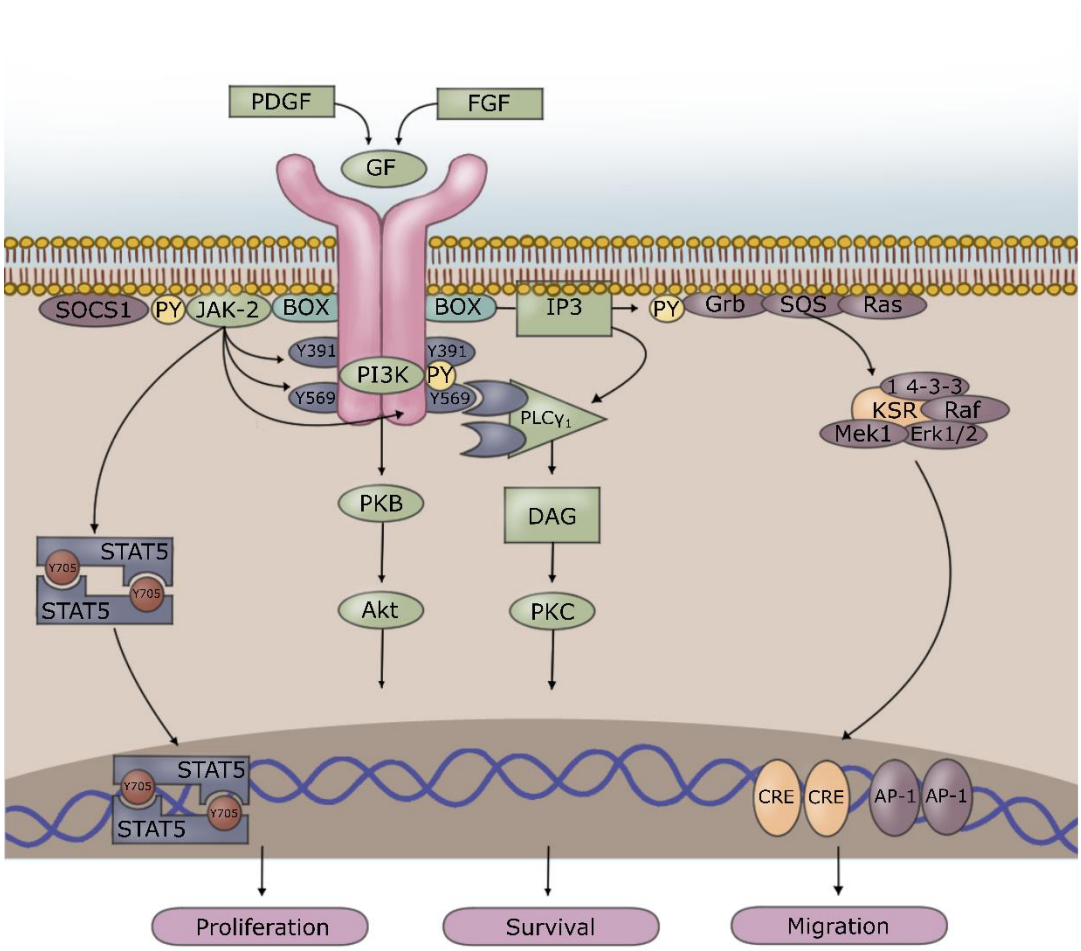

***Figure 3**: Growth factor signaling in PAH. Expression of certain growth factors is increased in PAH, promoting cell proliferation, survival, and migration via a number of signaling pathways, and thus contributing to pulmonary vascular remodeling. Abbreviations: see page 1*

Figure adopted with modifications from Schermuly et al. 2011

1.2.2 PDGF in PH pathophysiology

In lungs of PAH patients, an increased expression of mRNA of both PDGF-A and -B as well as PDGF receptors α and β could be demonstrated, the protein expression of PDGFR-β was increased as well. Histologically, PDGF-AA and -BB were found to be located in PASMCs and PAECs, PDGFR-α and β were expressed mainly in PASMC (Perros et al. 2008). PDGF-DD expression was not examined in this study. Schermuly et al. also reported a significantly increased protein expression of PDGFR-β and its phosphorylated form in lungs of patients with idiopathic PAH (Schermuly et al. 2005). Further, pulmonary arterial blood taken from PAH patients was shown to contain higher concentrations of

PDGF-BB compared to healthy controls or to systemic blood from patients and controls (Selimovic et al. 2009). An increase of PDGF-BB and PDGFR-β expression has also been found in monocrotaline (MCT)-induced PAH in rats and hypoxia-induced PAH in mice. The involvement of PDGF-BB and PDGFR-β in the pathogenesis of PH is supported by PDGFR-inhibition. Schermuly et al. treated animals from the abovementioned PH models with Imatinib (STI571), a tyrosine kinase inhibitor (TKI) that specifically inhibits BRC-ABL, c-kit and PDGFR-α and β, and could show attenuated remodelling of pulmonary arteries and a decreased expression of PDGF-BB and PDGFR-β (Schermuly et al. 2005). Further, hemodynamic parameters also improved due to PDGFR-inhibition by imatinib, shown by the reduction of the pulmonary arterial pressure (Schermuly et al. 2005). In vitro, Imatinib was also shown to inhibit the PDGF-BB induced proliferation and migration of PASMC from PAH patients (Perros et al. 2008) as well as the proliferation of rat PASMC treated with MCT (Schermuly et al. 2005). Recently, there is growing evidence that PDGF also regulates the vascular tone and possibly also influences this aspect of PH. This assumption is based on data which show a pulmonary vasorelaxant effect of Imatinib in isolated rat PA as well as the improvement of pulmonary hemodynamics in rats with MCT-induced PH (Abe et al. 2011; Pankey, Thammasiboon, et al. 2013). Preliminary results from our group show that imatinib relaxes ET-1 pre-constricted pulmonary veins (PVs) in precision cut lung slices (PCLS) of guinea pigs and that PDGF-BB contracts PVs, an effect which was prevented by the TKI Imatinib (Maihöfer et al. 2017; Rieg et al. 2018). These results suggest that the essential role of PDGF and PDGFR in PAH/PH pathogenesis is not only based on their proliferative properties, but also on their effects within the regulation of the pulmonary vascular tone. Consequently, PDGFR antagonism might be useful in PAH/PH therapy.

1.2.3 PDGFR antagonism by TKIs: Imatinib

In addition to the results mentioned above, there have been some some case reports of improved PAH in patients with chronic myelogenous leukemia (CML) receiving Imatinib treatment. Thus, the IMPRES study, a randomized controlled trial, has been conducted to test the benefit and safety of Imatinib therapy in PAH. This study included 202 patients with PAH FC III and IV and a PVR > 800 dynes x s x cm^{-5}, who were suffering from

symptoms in spite of already receiving at least two specific PAH therapies. For these patients, an additional treatment with Imatinib for 24 weeks caused a significant increase of the 6MWD and improved pulmonary haemodynamic parameters. However, no significant difference in FC or mortality were observed compared to the placebo group (Hoeper et al. 2013). These findings suggest a promising potential of Imatinib in the therapy of severe PAH, but lately data from a follow-up study suggest severe adverse effects due to Imatinib which increased mortality. Among the adverse effects are subdural hematoma, syncope and pleural effusion (Frost et al. 2015). Further, single cases of reversible selective pulmonary vascular toxicity due to Dasatinib, an unspecific TKI, have been reported (Godinas et al. 2013). Further research will be necessary to assess the efficacy and safety of Imatinib and different TKIs as a clinical drug in PAH therapy. There is evidence suggesting that not only the anti-proliferative effects but also the vasorelaxant effects of TKIs depend on PDGFR antagonism (Abe et al. 2011; Pankey, Thammasiboon, et al. 2013; Rieg et al. 2019). Thus, the effects of PDGFR agonism and antagonism on pulmonary vascular tone need to be examined more closely.

2. Goals and objectives

PH still has a poor prognosis due to insufficient therapeutic options. A deeper understanding of the underlying pathogenic mechanisms is essential to finding effective therapies. The role of PDGF and PDGF receptors in PAH has been emphasized both by the increased expression in PAH models and patients, as well as by the reversing and preventive effect of Imatinib in PAH-patients and chronic PAH-animal models. Data concerning the regulation of the pulmonary vascular tone by PDGF-BB/PDGFR has been obtained using guinea pigs, rats and human pulmonary tissue (Abe et al. 2011; Maihöfer et al. 2017; Rieg et al. 2018; Sachinidis et al. 1990). Presently, the total extent and the molecular mechanisms of the relationship between pulmonary vascular tone and PDGF-BB/PDGFR are not fully understood, and have not yet been examined in murine lungs, except for pre-published data from this study (Rieg et al. 2019). This study examines the effect of PDGF-BB and PDGF-CC on the tone of murine pulmonary vessels and the mechanisms behind the effect of PDGF-BB in order to find new ways of intercepting pathogenic signalling pathways. Furthermore, this study aims to investigate a possible vasodilating effect of Imatinib in murine pulmonary vessels with and without pre-constriction.

Without doubt, human data are of the highest impact, however difficult to generate and collect. Besides human tissue, mice are important in PAH research, as several chronic animal models are available (MCT, hypoxia, hypoxia/VEGF and PA-banding) (Ciuclan et al. 2011; Van et al. 2014). Moreover, the availability of genetic knock-out and knock-down models provides a convenient way to look further into molecular disease mechanisms. While knock-out technology in rats is increasingly common, a vast extent of experience is available for mice. Therefore, mice are still one of the most relevant animal models.

PCLS are a promising research method that has already generated a lot of valid data in several animal models and human tissue. Data on its application on murine tissue, with regard to the pulmonary vessels in particular, is limited. Thus, we not only aim to further the knowledge about the role of PDGF in the regulation of pulmonary vascular tone but also to examine and increase the usefulness of the mouse model and the PCLS method for future research in the field of PAH/PH.

3. Materials & methods

3.1 Devices

For the preparation of the murine slices two sets of tools were used, each comprising two pincers, two scissors and a tracheal canule. Agarose was kept at 38°C in a tempered water chamber (Julabo, type ED-19A). The lungs were sliced with a modified self-built Krumdieck tissue slicer. Work on the slices was performed under a LaminAir HB2448 clean bench by Heraeus Instruments. The slices were kept in a Binder incubator.

Effector and inhibitor substances were prepared with Sartorius analysis scales and a vortexer model VF2 by Janke & Kunkel as well as a Biozym centrifuge of the Sprout™ model.

3.2 Chemicals

Table 2: *Chemicals*

Chemical	**Manufacturer**
Agarose (1,5% and 3 %)	Biozym LE Agarose Gerbu Agarose LM
Ethanol 70%	Merck
H_2O (RNase free)	Macherey-Nagel
Pentobarbital (Narcoren, 16 mg/100 mL)	Merial GmbH

Table 3: *Cutting medium*

Component	**Concentration**
Calcium chloride (CaCl)	1,8 mmol/l
Magnesium sulfate ($MgSO_4$)	0,8 mmol/l
Potassium chloride (KCl)	5,4 mmol/l
Sodium chloride (NaCl)	116,4 mmol/l
Monosodium phosphate (NaH_2PO_4)	1,2 mmol/l
Glucose	16,6 mmol/l
Sodium hydrogen carbonate ($NaHCO_3$)	26,2 mmol/l
HEPES* (4-(2-hydroxyethyl)-1-peperazineethanesulfonic acid)	25,5 mmol/l

Table 4: *Additional components in P/S-medium.*

Medium	**Components and Concentration**
Sodium Pyruvate	1,2 mmol/l
MEM Amino Acids	20 ml/l
MEM Vitamins	10 ml/l
Glutamin	100 µmol/l
Penicillin	1% (v/v)

Streptomycin	1% (v/v)

Table 5: *Overview of all used inhibitors with targets, respective IC_{50} and concentration used in experiments.*

Inhibitor	**Target**	**IC_{50}**	**Conc.**
Imatinib			
(Medarametla et al. 2014)	PDGFR	0,6 µmol/l	≤ 100 µmol/l
(Heinrich et al. 2000)	c-Kit	0,1 µmol/l	
(Manley et al. 2010)	DDR1	0,045 µmol/l	
(Manley et al. 2010)	DDR2	0,15 µmol/l	
(Deininger and Buchdunger 2005)	BCR-ABL1	0,5 µmol/l	
Wortmannin			
(Yano et al. 1993)	PI3K	2-4 nmol/l	100 nmol/l
(Liu et al. 2005)	PLK1	5,8 nmol/l	
(Sarkaria et al. 1998)	DNA-PK	16 nmol/l	
(Sarkaria et al. 1998)	ATM	150 nmol/l	
(Nakanishi et al. 1992)	MLCK	170 nmol/l	
AS252424			
(Pomel et al. 2006)	PI3Kγ	33 nmol/l	500 nmol/l
(Pomel et al. 2006)	PI3Kα	935 nmol/l	

GSK 1059615			
(Carnero 2009)	PI3Kα	2 nmol/l	100 nmol/l
(Carnero 2009)	PI3Kβ	0,6 nmol/l	
(Carnero 2009)	PI3Kδ	2 nmol/l	
(Carnero 2009)	PI3Kγ	5 nmol/l	
(Carnero 2009)	mTOR	12 nmol/l	
Amlodipin besylate			
(Y. J. Lee et al. 2011)	LTCC	1,9 nmol/l	100 nmol/l
ODQ			
(Garthwaite et al. 1995)	Guanylyl cyclase	20 nmol/l	1 µmol/l
L-NAME			
(Furfine et al. 1993)	eNOS (human)	39 nmol/l	100 µmol/l
(Furfine et al. 1993)	iNOS (murine)	4,4 µmol/l	

3.3 Animals

For the experiments, 72 BALB/c mice (20 ± 3 g) from Charles River (Sulzfeld, Germany) were used. All experiments were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (ID 84-02.04.2013.A146; 8.87-51.05.20.10.245). Care and housing conditions of the animals complied with the regulations of the European Parliament and the Council Directive on the protection of animals used for scientific purposes (2010/63/EU) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Pelleted food and germ-free water were accessible at all times. A 12-hour light-dark rhythm was established by artificial lighting. The cage was renewed at least twice a week.

3.4 Preparation of murine Precision Cut Lung Slices (PCLS)

The mice were given intraperitoneal injection of 160 mg/kg pentobarbital (Narcoren; Garbsen, Germany) for terminal narcosis. Afterwards, all four extremities were attached to an underlying pad and an incision of the skin was made from the pubic bone to the lower jaw. The abdomen was opened with pincers and scissors from the pubic bone to the sternum and cut further horizontally along the edge of the lower ribs (Fig. 4 A). The resulting flaps were pinned to the pad to maintain access to the abdomen. Then the liver was pulled towards cranial and the V. portae and the A. hepatica communis were severed to exsanguinate the animal. The cervical fasciae were opened and the thyroid gland dissected to reveal the trachea. Afterwards, a horizontal incision was made at the level of the 3rd to 5th tracheal cartilage and the trachea was cannulated with a steel tube. Then the abdominal diaphragm was opened in order to cause a collapse of the lung and to visually check the filling of the lungs with agarose. Next, the lungs were filled via the steel tube with 1,3 ml liquid 1,5% agarose gel pre-warmed to 38°C. Then, to cool and solidify the agarose gel the thorax was covered with ice for 20 min. Afterwards, the thoracic wall was opened along the sternum with blunt scissors to avoid injury to the lungs and pulled wide open by pinning it to the pad (see Fig. 4 B). The clavicles were cut and the trachea, the pulmonary vessels, the main bronchi as well as the connective tissue between lung and thorax were severed to extract the lungs (Fig. 4 C, D). After removal of the lungs, they were put separately in cylindrical plastic vials with a volume of 1,8 ml, embedded in liquid 3% agarose gel solution and put on ice for 20 more minutes to solidify the agarose gel in the tube. After that, the cap and bottom of the vessels were cut off in order to extract the gel cylinders with the lungs. Next, the gel cylinders were placed in a slicing machine (modified Krumdieck tissue slicer, Fig. 4 E) filled with cutting medium and cut into slices of 250 +/- 50 µmol/l thickness by steel blades which were changed after each lung. Afterwards, the slices were transferred into incubation P/S-medium and incubated at 38°C in an atmosphere of 5% CO_2 in petri dishes (Fig. 4 F). To wash out the agarose and remove the cell debris the medium was changed every 30 min for two hours and then every 60 min for three hours. Then the slices were left in the incubator for 24 hours. After that, the medium was changed once every day. For the experiments, the slices were evaluated microscopically and the ones showing both

intact pulmonary artery and pulmonary vein with visible lumen in transverse section were selected and transferred to a 24 well plate in 1 ml or 500µl of fresh medium respectively.

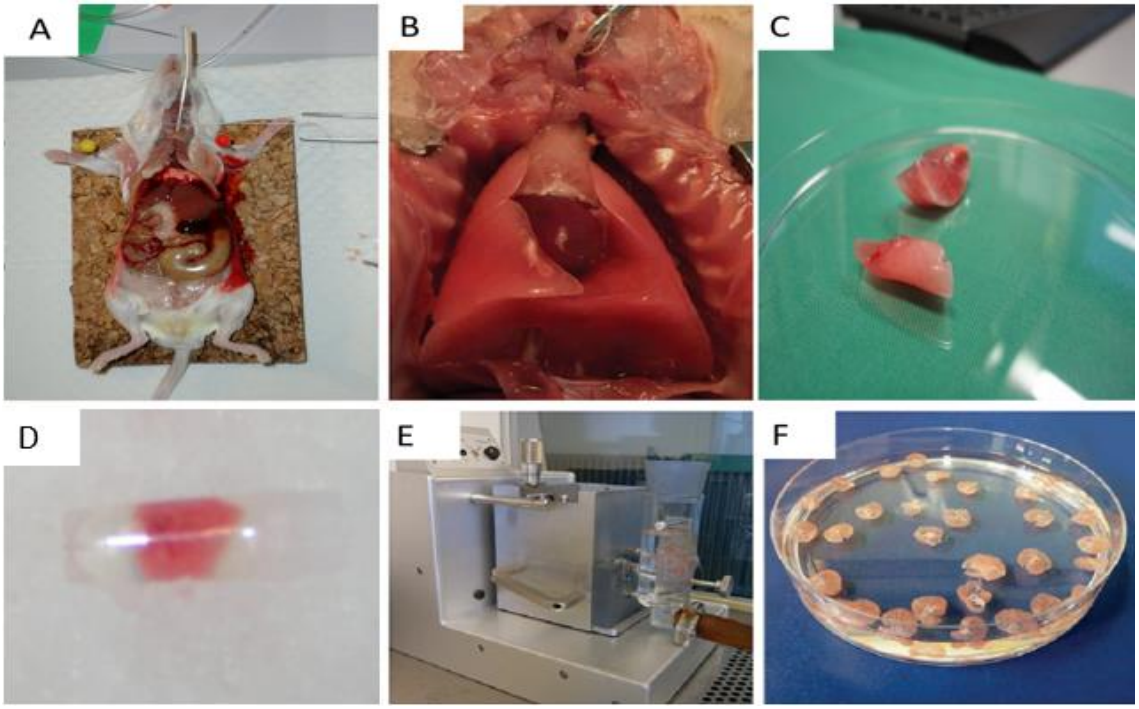

***Figure 4**: Preparation of precision-cut lung slices (PCLS). A: After anaesthesia and tracheotomy the lung lies collapsed in the thorax. B: Low-melting agarose (1.5 % (w/v)) is filled into the lung via the trachea and solidified on ice. C: The lungs are removed separately from the animal. D: The lungs are prepared for the slicing by embedding in vials with agarose which solidifies and is extracted. E: PCLS are cut from the cores by means of a modified self-build Krumdieck tissue slicer. F: PCLS are cultured and are viable for at least 72 h*

Pictures and legend adopted with modifications from Schlepütz et al., 2011.

3.5 Identification of pulmonary vessels

Based on literature, murine PAs and PVs can be distinguished mainly by their respective position and distance relative to the airways and to the medial interlobar fissure of the lung. The PA is generally described as running close to the airways while the PV is situated in the interlobar septa, usually at a greater distance to the airways (Hunt et al. 2013; Savai et al. 2005).

During videomicroscopy of the PCLS we assigned the pulmonary vessels either to PA or to PV according to their anatomical landmarks. The airways could be safely identified by the moving kinocilia on the luminal side of the epithelial cell layer. One of the vessels usually ran along the bottom of the interlobar fissure. We identified this vessel as the pulmonary vein (PV). The other vessel ran on the opposite side of, and situated closer to, the airways. We identified this vessel as the pulmonary artery (PA). Size and shape of the vessels varied and were not necessarily specific for one or the other type of vessel (Figure 5).

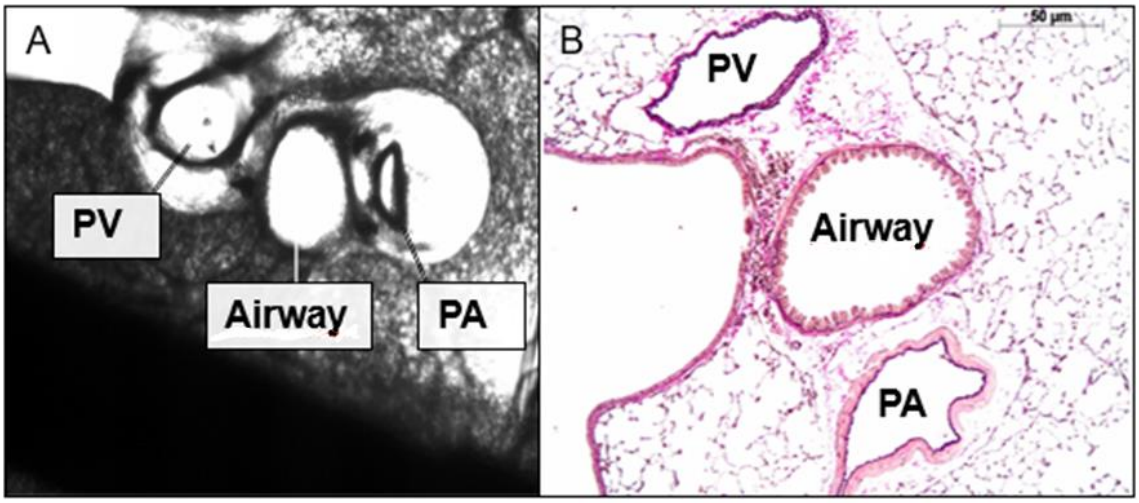

Figure 5: *A: Videomicroscopic picture of murine PCLS. Lung vessels and airway in transversal view. PV: Note position between airway and medial pulmonary interlobar edge, slightly longer distance to airway and less pronounced area of agarose gel expansion (white) in immediate vicinity. PA: Note position on the far side of airway relative to medial edge, shorter distance to airway and larger area of expanded agarose. B: Histologically stained picture of PCLS. PA: Note thick muscular layer. PV: Note two elastic layers. Picture B has been pre-published with alterations* (Rieg et al. 2019).

We verified the videomicroscopical assignment of the vessels using histological analyses of PCLS. We found that the vessel we identified as the PV has no obvious muscular media layer but two elastic layers similar to the membrana elastica interna and externa of an artery. At the same time the vessel we identified as the PA has a thick muscular tunica media characteristic for an artery, but only one discernible elastic layer. Thus, both vessels seemed to combine histological features of arterial and venous vessels.

We further controlled our vessel assignment by an additional method. In this trial we attempted to distinguish the PA from the PV by the use of coloured agarose. After deflating the lungs we opened the thorax and carefully injected 1,5% agarose gel coloured with toluidine blue into the main pulmonary artery. When a resistance could be sensed, presumably because the gel had filled the pulmonary arterial system all the way to the capillaries, the injection was stopped. After the gel had been solidified by cooling, the lungs were removed and embedded in an agarose gel cylinder as described in 2.4. A horizontal cut was made through this cylinder to expose both the PA filled with coloured gel and the empty PV, which were macroscopically discernible. Next, the cylinder was cut vertically in order to separate PA and PV. Both parts were labelled and the histological structures of the respective vessels analysed by the Institute of Pathology of UKA (Picture B). Univ.-Prof. Dr. med. R. Knüchel-Clarke, head of the Institute of Pathology of UKA, kindly agreed to review our findings. She confirmed that what we had identified as PV, with two elastic layers and barely any muscular layer, is indeed the PV, and that what we had identified as the PA, with a thicker muscular layer and only one discernible elastic layer, is indeed the PA. It is possible, however, that the second elastic layer of the PA is compressed by the muscular tunica media.

3.6 Videomicroscopy

The culture plates with the slices were placed under a Leica DMIL microscope and the 100x magnified picture captured by either a Leica DFC 295 or Leica Viscam 1280 camera was transmitted to a computer screen. The slices were immobilized by frames of platinum wire and nylon and positioned so that the PA, the PV and the airway were clearly and completely visible on the screen. After each use the platinum frames were

washed first with 70% Ethanol and then with distilled water. Increasing concentrations of the investigated substances were added to the medium and pictures were taken by the camera at a rate of 1/30s, 1/60s or 1/90s during the incubation time of 10, 20, 30, 60 or 90 min. In PCLS, all changes of the initial vessel area (IVA) were quantified in % and reported as “Change [%] of IVA”. Hence, a vessel area <100% indicates contraction and a vessel area >100% indicates relaxation. To compare relaxation or contraction of pre-treated vessels, the IVA after pre-treatment were again defined as 100%. The IVA as well as its alterations over the time were measured with Optimas6.5 (Media Cybernetics, Bothell, WA) and ImageJ (open source image processing). Both programs were used to analyse and evaluate the IVA over the time, using manually set thresholds for signal intensity/brightness which were set at the beginning and maintained for each picture sequence. For further analysis, the IVA- and AWA-values were saved in excel-charts. The GraphPad Prism 5.0 software was used for creating graphs from the data.

3.7 Statistics

Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA) and SAS software 9.3 (SAS Institute, Cary, North Carolina, USA). The data in Fig. 9, Fig. 10 were analyzed using a linear mixed model analysis (LMM) with the covariance structures VC; EC_{50} values (Fig. 8, Fig. 11) were calculated by the standard 4-parameter logistic non-linear regression model (GraphPad, La Jolla, USA). The AIC-criterion was used to select the most parsimonious model, i.e. a common top, bottom, slope and EC_{50} value in the regression model or the covariance matrix with the least number of parameters. Non-parametric analysis was performed by the by the Mann Whitney U test (Fig. V66, Fig. 15, Fig. 16, Fig. 17, Fig. 18, Fig. 19, Fig. 20, Fig. 21) to compare the maximal contractile effects of PDGF-BB (V66) after and without pre-treatment with the appropriate inhibitors of the investigated signalling cascades and by the Wilcoxon signed-rank test (Fig. 14, Fig. 15, Fig. 16, Fig. 17, Fig. 18, Fig. 19, Fig. 20, Fig. 21) to compare the maximal contractile effect against the control (100% value). A p-value < 0.05 was considered significant. For all experiments, n is the number of animals. All values are presented as mean ± SEM.

Table 6: *Software*

Software	**Manufacturer**
GraphPad Prism 5 Software	GraphPad, San Diego, CA, USA
Statistical Analysis Software	SAS Institute, North Carolina, USA
Optimas 6.5	Media-Cybernetics, Rockville, MD, USA
Image Lab Software	Bio-Rad Laboratories GmbH, Munich, Germany

3.8 Experiments with effector and inhibitor substances

First, a control trial was done in which the naïve PCLS were incubated with medium and did not receive any treatment for 60 min, during which pictures were taken at a rate of 30^{-1} Hz. Due to the lack of a significant change the vessel area and the small number of acceptable PCLS we refrained from running naïve control trials in the further trials.

Imatinib: To evaluate the vascular effect of imatinib, concentration-response curves from 100 pmol/l to 100 µmol/l were performed. This was first done in naïve PCLS with an incubation time of 20 min per concentration and a picture rate of 15^{-1} Hz before extending the experiments to PCLS pre-constricted with Endothelin-1 (ET-1), a nonselective ET-1 receptor agonist. ET-1 in concentrations of 100 nmol/l and 1 µmol/l were used for 60 min before adding Imatinib with an incubation time of 20 min for each concentration and a picture rate of 30^{-1} Hz.

In advance to these trials, the effect of ET-1 on the IVA was studied in a concentration-response curve from 100 pmol/l to 10 µmol/l, and in single concentrations of 100 nmol/l and 1 µmol/l for 90 min respectively, with pictures taken at a rate of 60^{-1} Hz.

In another trial, naïve slices were incubated with Imatinib in a concentration of 1 µmol/l for 120 min, pictures were taken at a rate of 60^{-1} Hz.

According to ET-1, a concentration-response curve of PDGF-BB was measured from 100 fmol/l to 100 nmol/l with an incubation time of 20 min per concentration and a picture rate of 30^{-1} Hz. After determining that a concentration of 100 nmol/l causes significant vasoconstriction in PAs, the further experiments were done with 100 nmol/l PDGF-BB, as the application of 10 nmol/l had no relevant contractile effect in murine PCLS. In these experiments, PCLS were treated with 100 nmol/l PDGF-BB for 30 min and pictures were taken at a frame rate of 60^{-1} Hz.

According to PDGF-BB, concentration-response curves were made for the PDGF ligand dimer PDGF-CC. Similar to PDGF-BB, concentrations of 100 pmol/l to 100 nmol/l were tested with a respective incubation time of 20 min for each concentration and pictures taken at a rate of 30^{-1} Hz.

In order to study whether the contractile effect of PDGF is linked to PDGFR, PCLS were pre-treated for 60 min with 1 µmol/l Imatinib before adding 100 nmol/l PDGF-BB. The change of IVA was then observed for an incubation time of 30 min at a picture rate of 60^{-1} Hz. For each trial, a control trial was run with PCLS taken from the same mouse which were treated only with 100 nmol/l PDGF-BB without having undergone pre-treatment with Imatinib, also observed over 30 min at a picture rate of 60^{-1} Hz.

In order to study the mechanisms beyond PDGF-BB induced contraction in PCLS, various signalling pathways were inhibited. Therefore, PCLS were pre-treated with the appropriate inhibitors, e.g. with 100 nmol/l Wortmannin, an unspecific inhibitor of PI3-K, 100 nmol/l GSK 1059625 (PI3-K α), 500 nmol/l AS 252424 (PI3-K γ), 100 nmol/l Amlodipin (L-type Ca2+-channels), 100 µmol/l L-NAME (NO) and 1 µmol/l ODQ (sGC). PCLS were incubated with one of these inhibitors for 60 min prior to the adding of PDGF-BB in a concentration of 100 nmol/l. The IVA was measured for 30 min at a picture rate of 60^{-1} Hz. For each trial, controls were run with PCLS taken from the same mouse. Those control PCLS received treatment with 100 nmol/l PDGF-BB without the inhibitor.

In order to assess whether PDGF-BB induced vasoconstriction depends on PDGFR-α or PDGFR-β, PCLS were pre-treated with 100 nmol/l Ponatinib (PDGFR-α-inhibitor) or 5

µmol/l SU6668 (PDGFR-β-inhibitor) for 60 min prior to the application of 100 nmol/l PDGF-BB. Pictures were recorded at 60^{-1} Hz. A control trial was performed with only PDGF-BB for 60 min.

4. Results

In PCLS, increasing concentrations of Imatinib (0.1 nmol/l – 10 µmol/l) did not alter the IVA of naïve murine PAs and PVs (Fig. 6). Therefore, we used ET-1, an ET-1_A receptor agonist, to achieve pre-contraction of the pulmonary vessels. PAs and PVs of PCLS responded with equal vasoconstriction to the increasing concentrations of ET-1 (0,1 nmol/l – 10 µmol/l) (Fig. 7). The EC_{50} could be determined at 125 nmol/l (Fig. 7). ET-1 at 100 nmol/l and 1 µmol/l caused a lasting and significant vasoconstriction over 90 min of both PAs and PVs (Fig. 8, 9). The decrease of IVA was more prominent for 1 µmol/l ET-1, however the contractile effect in PAs and PVs did not differ statistically (Fig. 8, 9).

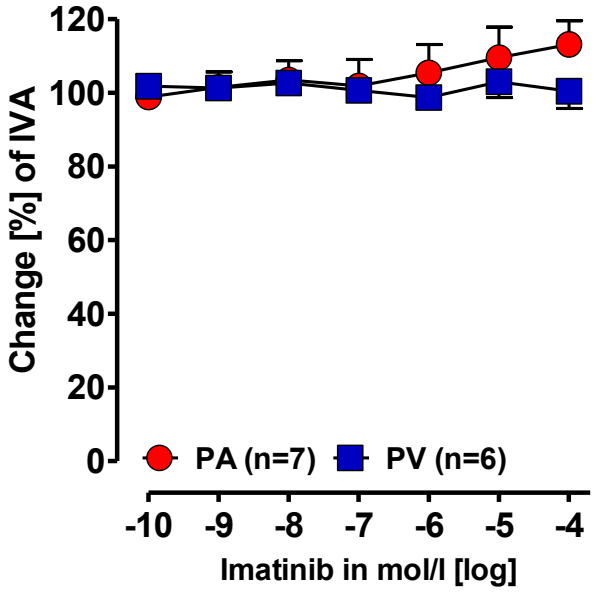

Figure 6: *Change of IVA of PA (red data plot) and PV (blue data plot) in response to increasing concentrations of Imatinib (0,1 nmol/l - 100 µmol/l). Data is Mean ± Standard Error of the Mean (SEM).*

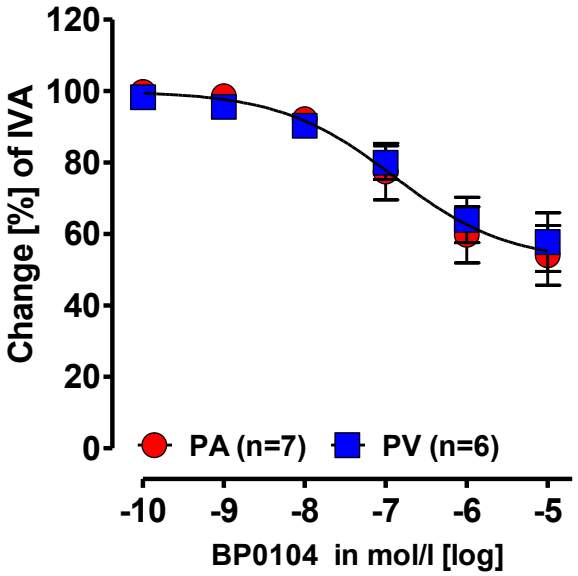

Figure 7: *Change of IVA of PA and PV in response to increasing concentrations of ET-1 (0,1 nmol/l - 10 µmol/l). Data is Mean ± SEM. Statistical analysis: calculation and comparison of* EC_{50} *values and maximum change of IVA. Figure has been pre-published with alterations* (Rieg et al. 2019).

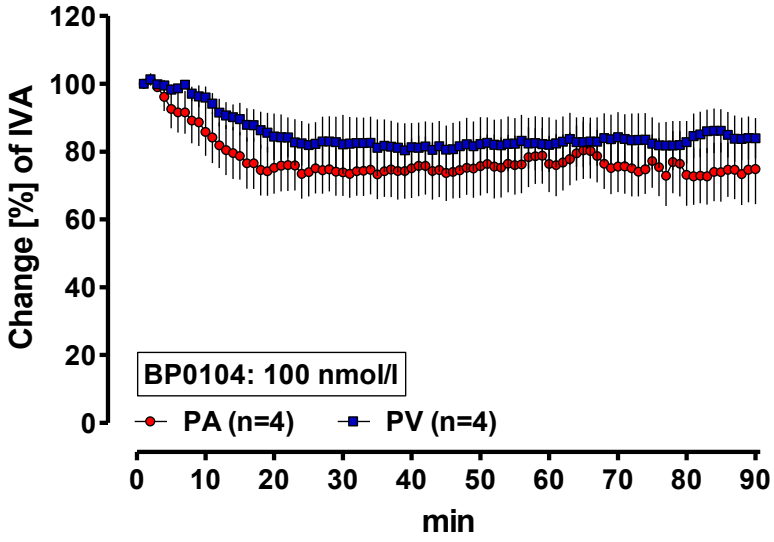

Figure 8: *Change of IVA of PA and PV in response to incubation of PCLS with 100 nmol/l ET-1 for 90 min. Data is Mean ± SEM. Statistical analysis: SAS linear mixed model. Figure has been pre-published with alterations* (Rieg et al. 2019)*.*

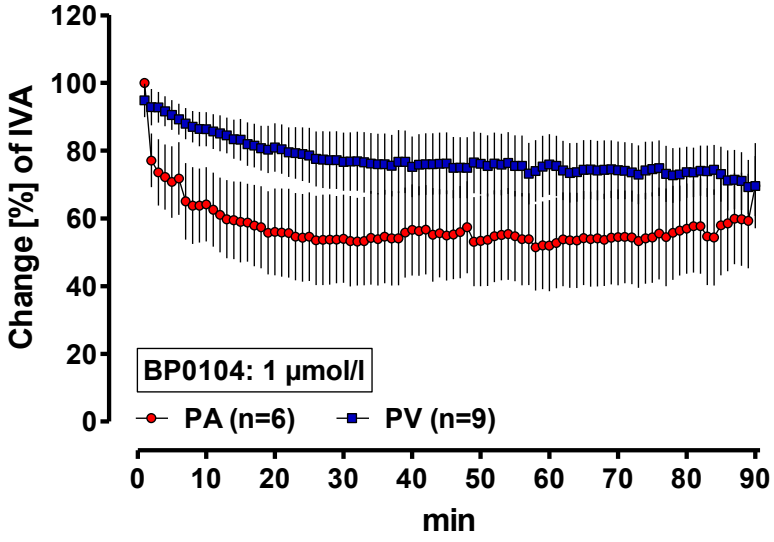

Figure 9: *Change of IVA of PA and PV in response to incubation of PCLS with 1 µmol/l ET-1 for 90 min. Data is Mean ± SEM. Statistical analysis: SAS linear mixed model.*

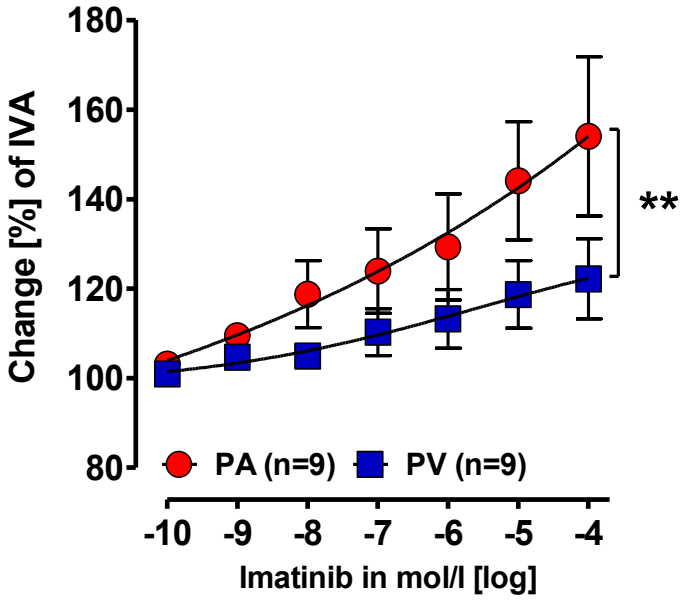

Figure 10: *Change of IVA of PA and PV in response to increasing concentrations (0,1 nmol/l - 100 µmol/l) of Imatinib after 60 min incubation with 1 µmol/l ET-1. Data is Mean ± SEM. Statistical analysis: calculation and comparison of EC_{50} values and maximum change of IVA. Figure has been pre-published with alterations* (Rieg et al. 2019).

After ET-1 pre-constriction, Imatinib in increasing concentrations (0,1 nmol/l – 100 µmol/l) relaxed the PAs up to 154% of IVA with an EC_{50} of 4,9 µmol/l (Fig. 10). The PVs showed a vasorelaxation up to 122% (mean of maximal relaxation) of IVA with an EC_{50} of 1,2 µmol/l (Fig. 10). Finally, imatinib relaxed pre-constricted PAs stronger than pre-constricted PVs ($p<0.01$).

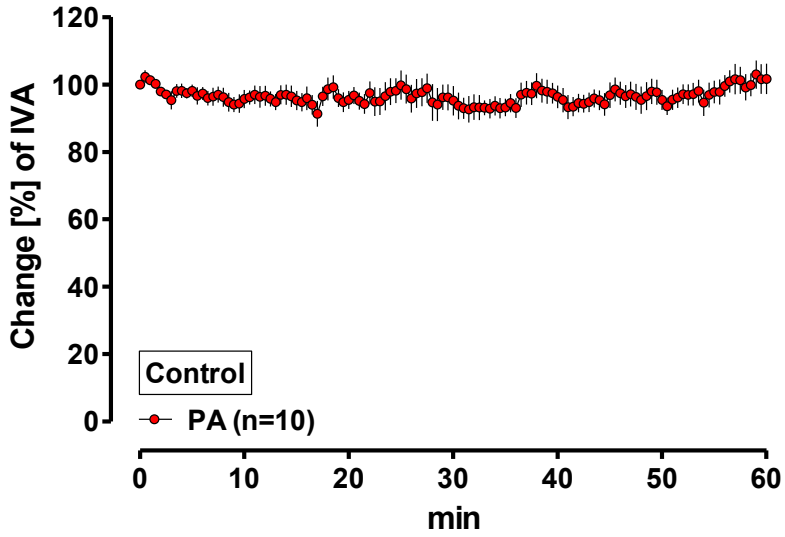

Figure 11: *Change of IVA of PA in response to incubation of PCLS with P/S medium for 60 min. Data is Mean ± SEM.*

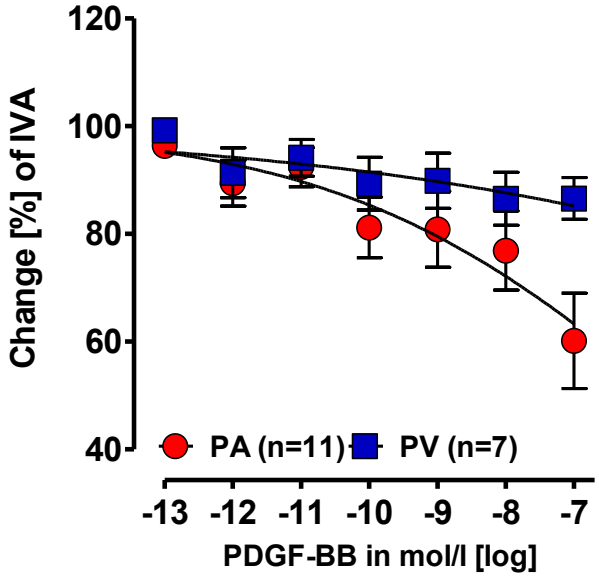

Figure 12: *Change of IVA of PA and PV in response to increasing concentrations of PDGF-BB (0,1 pmol/l – 100 nmol/l). Data is Mean ± SEM. Statistical analysis: calculation and comparison of* EC_{50} *values and maximum change of IVA. Figure has been pre-published with alterations* (Rieg et al. 2019).

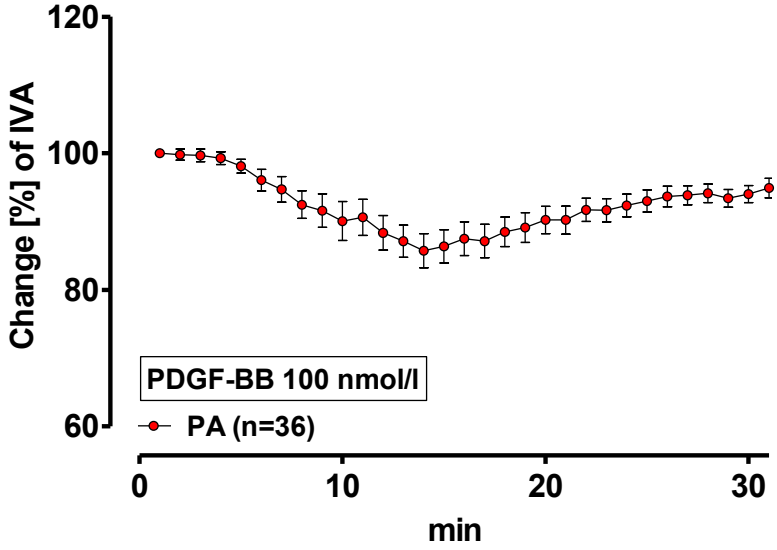

Figure 13: *Change of IVA of PA (red data plot) in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min. Data is Mean ± SEM. Statistical analysis was performed using Wilcoxon signed rank test. Figure has been pre-published with alterations* (Rieg et al. 2019).

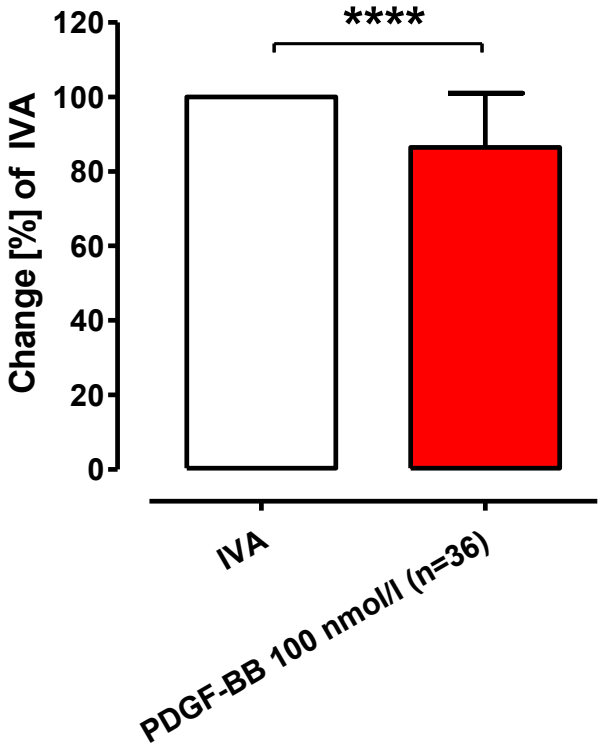

Figure 14: *Maximal change of IVA of the PA (red bar) in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min. White bar shows initial vessel area. Bars represent Mean + SEM. Statistical analysis was performed using the Wilcoxon-signed rank test. P < 0,05 was considered statistically significant.*

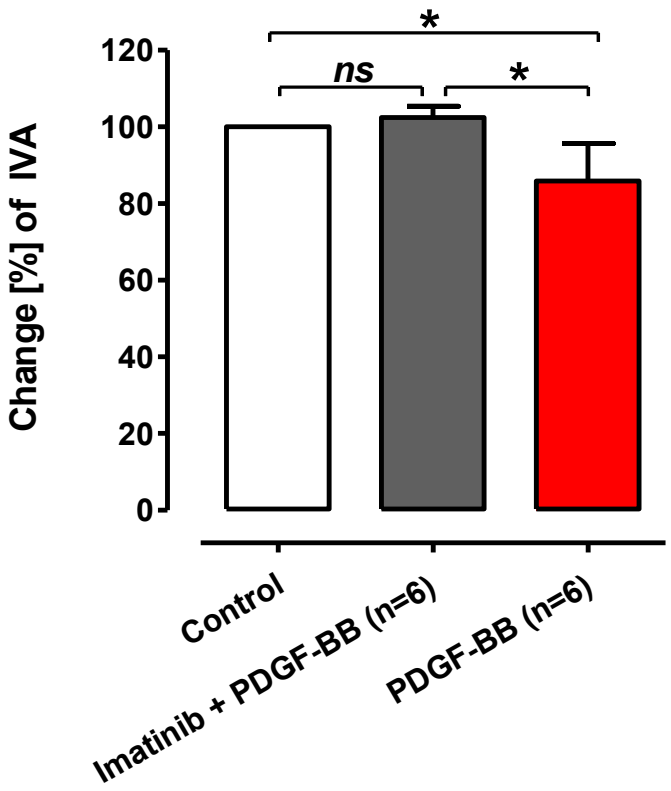

Figure 15: *Maximal change of IVA of the PA in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min after pre-treatment with 1 µmol/l Imatinib for 60 min (grey bar). Paired trials received incubation with 100 nmol/l PDGF-BB without pre-treatment (red bar). White bar shows initial vessel area. Bars represent Mean + SEM. Statistical analysis was performed using the Mann-Whitney U test (PDGF-BB vs. Imatinib /PDGF-BB) and the Wilcoxon-signed rank test (PDGF-BB vs. control and Imatinib /PDGF-BB vs. control). P < 0,05 was considered statistically significant.*

We found that the PA IVA of naïve PCLS did not change when the slices were just incubated in medium without any treatment (Fig. 11). Thus, in the following trials with PDGF-BB and inhibitors where only PAs were examined, the change of vessel area was statistically analysed in comparison to the IVA with no further control trials.

Increasing concentrations of PDGF-BB significantly ($p<0,0001$) contracted PAs to approximately 60% of IVA and the maximal effect was observed at the highest tested concentration of 100 nmol/l (Fig. 12). In contrast, PVs only contracted down to 86% of IVA at 100 nmol/l PDGF-BB (Fig. 12).

Because the effect was so weak in PVs, in the following trials we only examined the PAs.

Incubation with 100 nmol/l PDGD-BB for 30 min caused a significant vasoconstriction of PA up to 85% of IVA ($p<0,001$) (Fig. 13, 14). The contraction regularly reached its maximum within 20 minutes after incubation with 100 nmol/l PDGF-BB, and then gradually decreased (Fig. 13).

Pre-treatment with Imatinib in a concentration of 1 µmol/l completely prevented the vasoconstricting effect (Fig. 15).

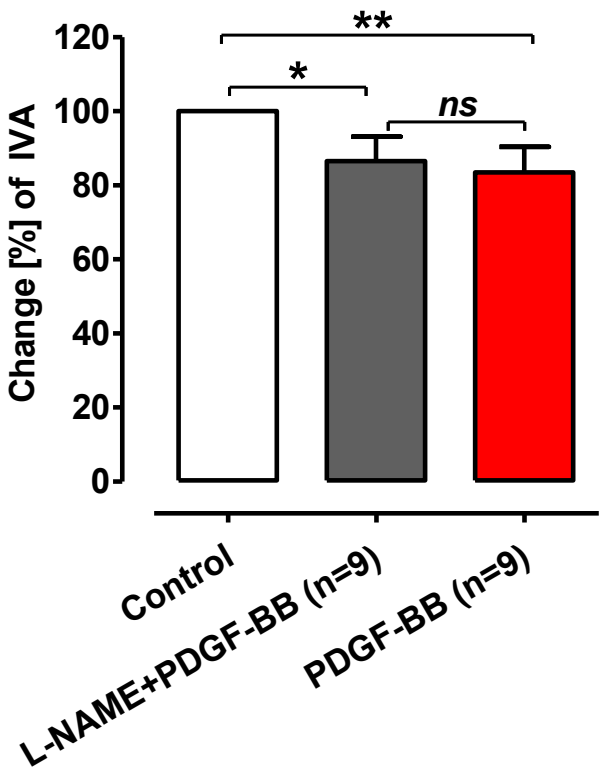

Figure 16: *Maximal change of IVA of the PA in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min after (grey bar) or without (red bar) pre-treatment with 100 µmol/l NG-nitro-L-arginine methyl ester (L-NAME) for 60 min. White bar shows initial vessel area. Bars represent Mean + SEM. Statistical analysis was performed using the Mann-Whitney U test (PDGF-BB vs. L-NAME/PDGF-BB) and the Wilcoxon-signed rank test (PDGF-BB vs. control and L-NAME/PDGF-BB vs. control) P < 0,05 was considered statistically significant.*

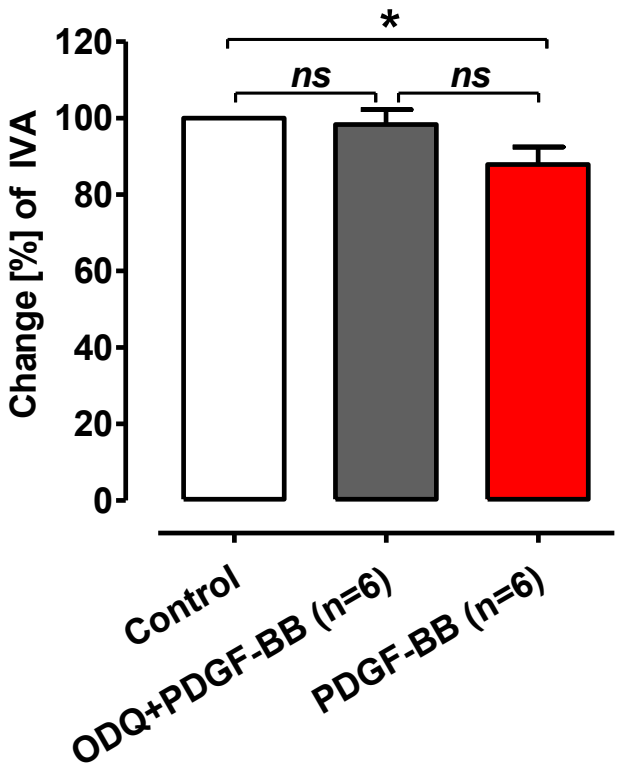

Figure 17: *Maximal change of IVA of the PA in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min after (grey bar) or without (red bar) pre-treatment with 1 µmol/l ODQ for 60 min. White bar shows initial vessel area. Bars represent Mean + SEM. Statistical analysis was performed using the Mann-Whitney U test (PDGF-BB vs. ODQ /PDGF-BB) and the Wilcoxon-signed rank test (PDGF-BB vs. control and ODQ /PDGF-BB vs. control). P < 0,05 was considered statistically significant.*

Inhibition of eNOS with L-NAME, a specific inhibitor of NO-synthase, did not significantly increase the vasoconstricting effect of PDGF-BB (Fig. 16). Moreover, pre-treatment with ODQ, an inhibitor of the sGC, did also not increase PDGF-BB mediated vasoconstriction (Fig. 17). However, somewhat unexpectedly, inhibition of sGC prevented the contractile effect of PDGF-BB (Fig. 17).

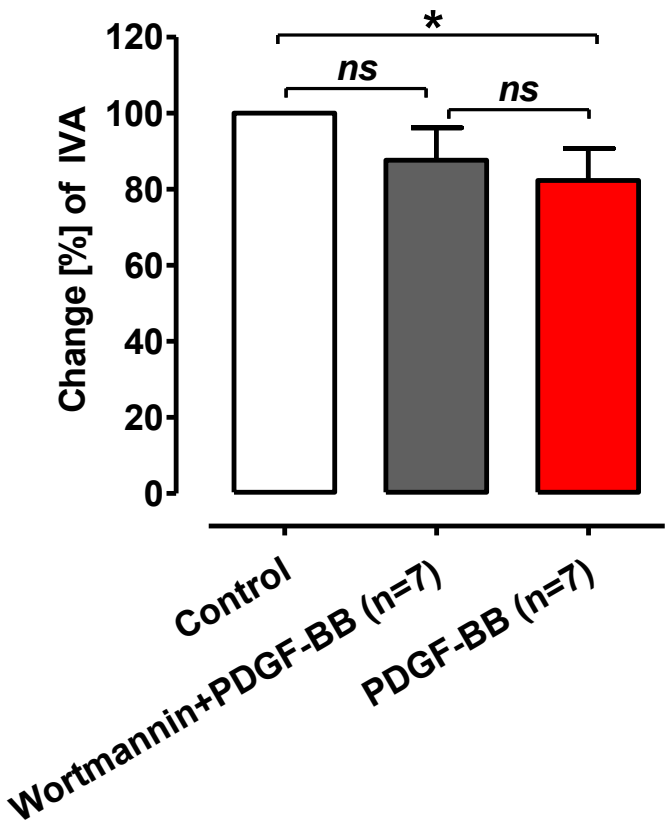

Figure 18: *Maximal change of IVA of the PA in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min after (grey bar) and without (red bar) pre-treatment with 100 nmol/l Wortmannin for 60 min. White bar shows initial vessel area. Bars represent Mean + SEM. Statistical analysis was performed using the Mann-Whitney U test (PDGF-BB vs. Wortmannin /PDGF-BB) and the Wilcoxon-signed rank test (PDGF-BB vs. control and Wortmannin /PDGF-BB vs. control). P < 0,05 was considered statistically significant.*

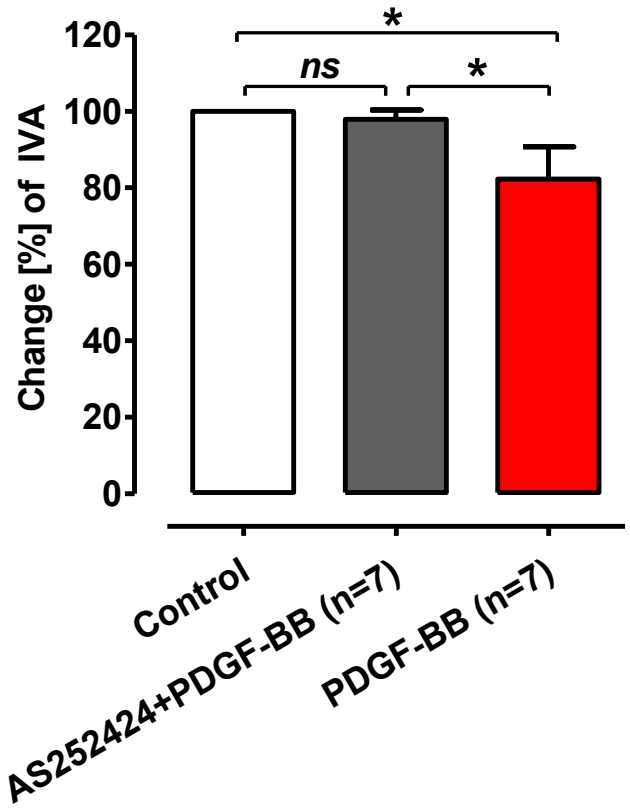

Figure 19: *Maximal change of IVA of the PA in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min after (grey bar) and without (red bar) pre-treatment with 500 nmol/l AS252424 for 60 min. White bar shows initial vessel area. Bars represent Mean + SEM. Statistical analysis was performed using the Mann-Whitney U test (PDGF-BB vs. AS252424 /PDGF-BB) and the Wilcoxon-signed rank test (PDGF-BB vs. control and AS252424 /PDGF-BB vs. control). P < 0,05 was considered statistically significant.*

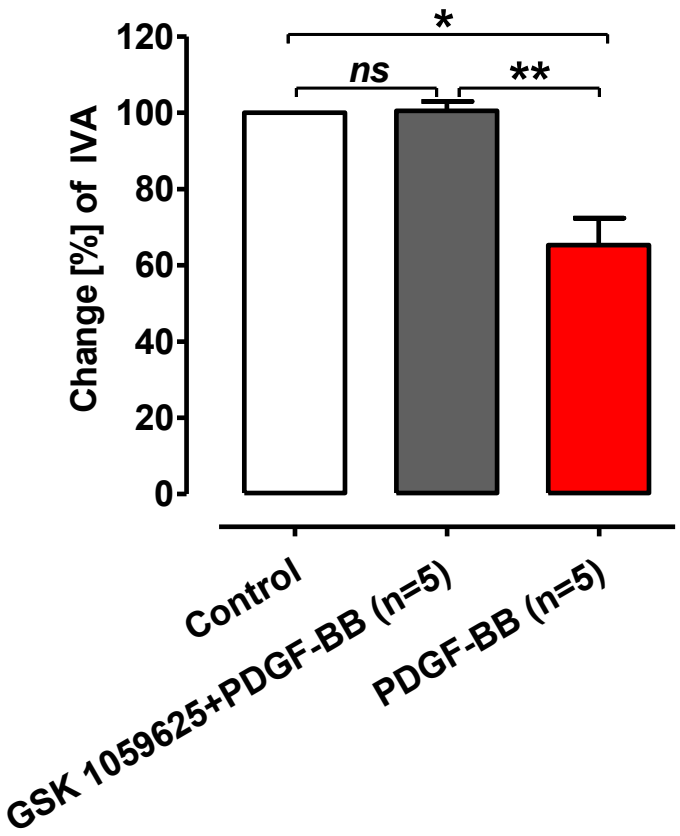

Figure 20: *Maximal change of IVA of the PA in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min after (grey bar) and without (red bar) pre-treatment with 100 nmol/l GSK1059615 for 60 min. White bar shows initial vessel area. Bars represent Mean + SEM. Statistical analysis was performed using the Mann-Whitney U test (PDGF-BB vs. GSK1059625 /PDGF-BB) and the Wilcoxon-signed rank test (PDGF-BB vs. control and GSK1059625 /PDGF-BB vs. control). P < 0,05 was considered statistically significant.*

In PCLS, pre-treatment with the nonselective PI3-K inhibitor wortmannin (100 nmol/l), did not significantly alter PDGF-BB-induced vasoconstriction compared to the treatment with PDGF-BB alone (Fig. 18). However, after pre-treatment with wortmannin the change of IVA was not significant anymore compared to control PAs (Fig. 18). The selective inhibition of PI3-K γ by pre-treatment with 500 nmol/l AS252424 significantly reduced the contractile effect of PDGF-BB in PAs (Fig. 19). In addition, selective inhibition of PI3-K α by pre-treatment with 100 nmol/l GSK1059615 also significantly decreased the vasoconstricting effect of PDGF-BB (Fig. 20).

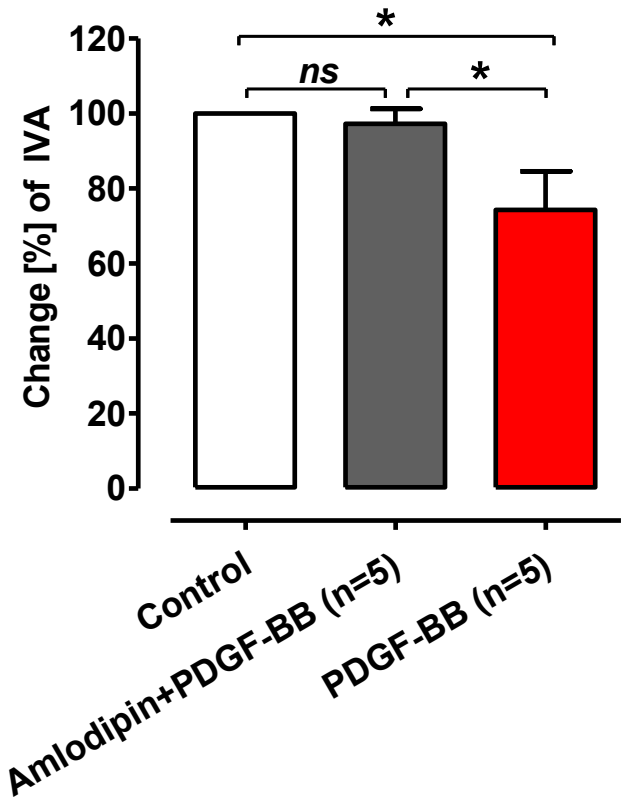

Figure 21: *Maximal change of IVA of the PA in response to incubation of PCLS with 100 nmol/l PDGF-BB for 30 min after (grey bar) and without (red bar) pre-treatment with 100 nmol/l Amlodipin for 60 min. White bar shows initial vessel area. Bars represent Mean + SEM. Statistical analysis was performed using the Mann-Whitney U test (PDGF-BB vs. Amlodipin /PDGF-BB) and the Wilcoxon-signed rank test (PDGF-BB vs. control and Amlodipin /PDGF-BB vs. control). P < 0,05 was considered statistically significant.*

Pre-treatment with 100 nmol/l Amlodipin, a selective L-Type Ca^{2+} channel inhibitor, significantly reduced the vasoconstricting effect of PDGF-BB (Fig.21).

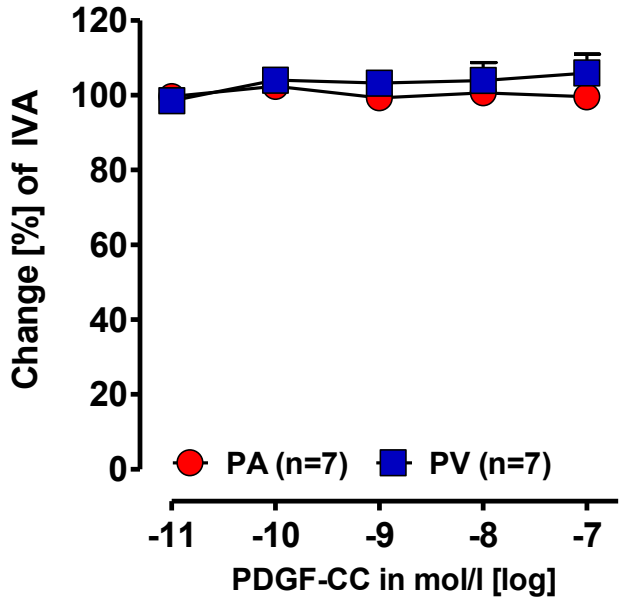

Figure 22: *Change of IVA of PA and PV in response to increasing concentrations of PDGF-CC (10 pmol/l – 100 nmol/l). Data is Mean ± SEM.*

PDGF-CC in concentrations up to 100 nmol/l had no significant contractile or relaxant effect on mouse pulmonary vessels (Fig. 22).

5. Discussion

5.1 Discussion of Results

PAH is a progredient disease with a poor prognosis. The pathogenetic mechanisms causing increased pulmonary arterial pressure (PAP) include remodelling of the pulmonary vessels and dysregulation of the vascular tone, both eventually leading to right heart failure and death.

Growing evidence suggests that the nonselective TKI Imatinib (STI571) is a promising new drug in PH therapy. Besides the antiproliferative effects, imatinib-induced vasorelaxation has been shown in rat PAs (Abe et al. 2011; Pankey, Thammasiboon, et al. 2013), guinea pig PVs (Maihöfer et al. 2017), and recently in humans and mice (Rieg et al. 2019). In addition, a contractile effect of PDGF-BB was proven in guinea pig PVs, humans and mice (Rieg et al. 2018, 2019). We designed this study with murine PCLS in order to find out whether the vasorelaxant effect of Imatinib and the contractile effect of PDGF-BB can also be observed in murine pulmonary vessels. The results have been partly pre-published (Rieg et al. 2019).

In murine PCLS, imatinib had a relaxant effect on pulmonary vessels pre-constricted with ET-1 that was significantly stronger in PAs than in PVs. Imatinib had no significant vasodilatory effect on naïve PAs and PVs. In line with these findings, many studies using different animal models observed that the vasorelaxant effect of imatinib is dependent on pre-constriction, which will be discussed later (Abe et al. 2011; Pankey, Thammasiboon, et al. 2013; Rieg et al. 2019). Further, imatinib is reported to lower the vascular tone in rat systemic arteries (Pankey, Lasker, et al. 2013) and rat cerebral vessels after subarachnoidal haemorrhage (Shiba et al. 2012) and to cause relaxation of prostatic smooth muscle cells (Ozgur-Akdemir et al. 2011). Hence, imatinib-induced vasorelaxation does not appear to be limited to pulmonary vascular SMCs.

The fact that imatinib relaxes murine PAs and PVs and that its counterplayer PDGF-BB enhances the pulmonary vascular tone in murine and human PAs and PVs and guinea pig PVs (Rieg et al. 2018, 2019) raises the interesting question whether and how PDGFR regulates pulmonary vascular tone. In this study we showed that 100 nmol/l PDGF-BB

contracts murine PAs to 60% of IVA and murine PVs to 86% of IVA. While the PVs were not further examined in this study due to the weak effect, in PAs this effect was prevented by pre-treatment with 1 µmol/l imatinib, suggesting specific agonism and antagonism at the PDGFR. In the following trials, we further analysed the underlying mechanism behind PDGF-BB-induced contraction of murine PAs. Our results suggest that the contractile effect of PDGF-BB depends on PI3K-α/-γ and LTCCs, whereas eNOS plays no role.

The role of eNOS and cGMP in PDGF-BB-induced vasoconstriction and vasorelaxation

The extent of PDGD-BB-induced vasoconstriction that we observed in the murine PCLS was relatively moderate. This led us to consider whether PDGF-BB might activate some vasorelaxant signalling pathway counteracting the vasoconstriction.

In guinea pig PVs, PDGF-BB-induced vasoconstriction is enhanced by additional inhibition of cAMP production and eNOS, suggesting a counteracting vasorelaxant effect via both pathways (Rieg et al. 2018). In human umbilical arteries and mouse aortic vessels PDGF-BB causes vasorelaxation via the PI3K-Akt-eNOS pathway, and in live mice there is a decrease of arterial pressure after PDGF-BB administration (Ha et al. 2011). This effect was also observed in rat aortic rings pre-constricted with Phenylephrin, whereas in endothelium-denuded vessels PDGF induces an eNOS-independent vasoconstriction that is stronger for PDGF-BB than for PDGF-AA or -AB (Cunningham et al. 1992). Takase et al. reported that PDGF causes a NO-dependent vasodilation in rat mesenteric arteries, which is attenuated in hypertensive rats (Takase et al. 1999).

Thus, we examined a possible NO-mediated vasorelaxant effect of PDGF-BB in murine PAs. L-NAME inhibits eNOS and leads to reduced cGMP and PKG-activity, which increases myosin-light-chain (MLC)-phosphorylation and SMC contractility. ODQ inhibits sGC directly.

We found that inhibition of eNOS by L-NAME did not significantly alter the PDGF-BB-induced contraction of murine PAs. Specifically, our results showed no enhanced PDGF-BB-induced vasoconstriction after pre-treatment with L-NAME, which makes an NO-

dependent counteractive effect of PDGF-BB unlikely. After sCG inhibition by ODQ, we could observe a decrease of the PDGF-BB-induced vasoconstriction that was, however, not statistically significant.

The literature data demonstrate the complexity of the interactions of NO and the vascular tone. There are both eNOS-dependent and independent mechanisms of PDGF-induced vasorelaxation in different kinds of vessels and in different species. Although murine pulmonary vessels express eNOS (Fagan et al. 2001), our results suggest that in murine PAs, PDGF-BB has no NO-mediated vasorelaxant effect that counteracts its vasoconstricting effect. The explanation could be that PDGFR activity does not affect eNOS expression and activity in murine PAs.

The questionable effect of ODQ raises the question whether PDGF-BB stimulates sGC independent of NO. To further examine the role of eNOS and cGMP (possibly independent of NO and in a vasoconstricting function) in PDGFR signalling and vascular tone, more experiments will be necessary. It will be interesting to examine the expression and activity of eNOS upon PDGFR activation, as well as sGC activity with and without inhibiton of NO, in murine pulmonary vessels, particularly PVs in which the PDGF-BB-induced vasoconstriction was much weaker. Further, a possible PDGF-BB-induced vasorelaxant effect mediated by cAMP, as has been found in guinea pig PVs (Rieg et al. 2018), should be examined in murine pulmonary vessels.

Moreover, it has been reported that PDGF-BB stimulates the expression of HIF-1α in the MCT-induced rat model of PAH (Cheng et al. 2019). While the role of HIF in PAH is generally considered a promoting one (Shimoda et al. 2019; Suresh and Shimoda 2016), the findings in regard to its effect on pulmonary vascular tone are controversial, and it has been demonstrated to have a vasorelaxing effect in mice (Kim et al. 2013). Whether HIF significantly contributes to the acute pulmonary vascular response to PDGF-BB remains to be seen.

The role of PI3K-α/γ in PDGF-BB-induced vasoconstriction

There is much evidence for PI3K playing an important role in PDGFR signalling and the regulation of vascular tone. In guinea pigs, PDGF-BB-induced vasoconstriction of PVs could be shown to be mediated via both PI3K-α and PI3K-γ (Rieg et al. 2018). Thus, we examined whether PDGF-BB-induced vasoconstriction in murine PAs is dependent on PI3K.

We found that, while both the specific PI3K-α-inhibitor GSK1059615 and the specific PI3K-γ-inhibitor AS252424 attenuate PDGF-BB-induced vasoconstriction, it is not affected by the non-selective PI3K-inhibitor wortmannin. This seems contradictory and could only partly be explained if pulmonary vasoconstriction in mice were mediated by PI3K-α isoform PI3K-C2-α which has been reported to have a vasoconstricting effect (Azam et al. 2007; Seok et al. 2010; Y. Wang et al. 2006) with a reduced sensitivity to wortmannin (Domin et al. 1997). It has not been examined yet whether PI3K-C2-α plays a role in the regulation of pulmonary vascular tone. However, other trials showed a successful antagonism of PI3K-C2-α by wortmannin (Seok et al. 2010). Further, in the case that inhibition of PI3K-α by wortmannin might be unsuccessful, the remaining inhibition of PI3K-γ could be expected to at least attenuate PDGF-BB-induced vasoconstriction to an extent similar to selective PI3K-γ-inhibitor AS252424. There is no evidence yet for a wortmannin-resistant isoform of PI3K-γ to explain that discrepancy. Whether such an isoform exists or whether PDGF-BB also has a PI3K-dependent vasorelaxant effect which becomes dominant when only one subtype is selectively inhibited would make an interesting subject for further research. Interestingly, a vasodilating effect of PDGF via not specifically identified PI3K, Akt/PKB and eNOS has been shown in the murine abdominal aorta (Ha et al. 2011).

One possible explanation for the observation that PDGF effectively causes both PI3K-dependent vasoconstriction and vasodilatation is that the balance of different PI3K-activated pathways is dependent on species, tissue and predominant PI3K subtype, as well as pathological changes of the environment, e.g. endothelium damage or PH. Further specific research is needed to clarify the roles of these factors and their

combinations in the various abovementioned effects and to determine the animal model most suited to simulate the changes and therapy of PH in human lungs.

The role of Ca^{2+} and LTCCs in PDGF-BB-induced vasoconstriction

We could show in this study that the PDGF-BB-induced vasoconstriction of murine PA was successfully inhibited by the LTCC-blocker amlodipine.

PDGF-BB has been shown before to cause Ca^{2+}-dependent vasoconstriction in the rat aorta (Berk et al. 1986; Sachinidis et al. 1990) and, most relevant to this study, in guinea pig PVs (Rieg et al. 2018). The PDGF-induced increase of intracellular Ca^{2+} and subsequent vasoconstriction is mediated by phospholipase C γ (PLC-γ)-dependent IP_3 and DAG release as well as increased activity of PKC (Block et al. 1989; Caglayan et al. 2011). It was observed that PDGF-BB has a stronger effect on IP_3 and DAG release and that PDGF-AA had a stronger effect on PKC activity and calcium sensitivity (Block et al. 1989). PDGF-AA causes vasoconstriction in vascular smooth muscle cells (VSMCs) without increasing intracellular Ca^{2+} (Sachinidis et al. 1990).

Interestingly, LTCC-blockers not only antagonize the vasoconstriction caused by Ca^{2+}-influx from extracellular but already inhibit the PDGF-induced release of IP_3 and DAG, and therefore the liberation of Ca^{2+} from the sarcoplasmatic reticulum (SR) (Block et al. 1989; Bornfeldt et al. 1995; Heldin et al. 1998; Rosenkranz and Kazlauskas 1999). The question whether and how an inhibition of LTCCs is able to intercept this step of the PDGFR signalling requires further research.

PI3K-γ-mediated vasoconstriction in rat systemic arteries is LTCC-dependent (Carnevale and Lembo 2012). This might be another way for PDGF to activate LTCCs in pulmonary vessels.

Other studies suggest that PDGF stimulates NADPH-oxidase in VSMCs, increasing ROS (Clempus and Griendling 2006), which have been shown to activate PKCε and increase

i(Ca^{2+}) via opening of RyR in the SR and inhibition of K+v, leading to depolarisation and opening of LTCCs (Y. X. Wang and Zheng 2010).

Our findings that PDGF-BB-induced constriction of murine PAs is dependent on LTCCs is in line with many other trials. The various ways in which LTCCs are activated, both by PDGFR and otherwise, are not exhaustively investigated yet.

The role of PDGF-CC on murine pulmonary vascular tone

We found that PDGF-CC, a PDGF subtype that binds specifically to PDGFR-α (Fang et al. 2004; Li et al. 2000), does not have a vasoconstricting effect on murine pulmonary vessels in PCLS in the concentrations that are effective for PDGF-BB (Fig. 11 B). PDGF-BB binds to both the PDGFR-α and PDGFR-β receptors, the latter receptor being specific for the PDGF-B monomer so that only PDGF-AB and PDGF-BB can cause its dimerization and activation (Fretto et al. 1993). These results suggest that while some effects of PDGF-BB may be mediated via PDGFR-α, the vasoconstrictory effect is likely to be mediated by PDGFR-β. This is in line with reports that specific inhibition of PDGFR-β attenuates the PDGF-BB induced vasoconstricion in guinea pig PV while specific inhibition of PDGFR-α does not affect the vasoconstriction (Maihöfer et al. 2017).

The relaxant effect of imatinib depends on pre-constriction of PAs and PVs

An observation that has not yet been explained sufficiently in this and other trials is that imatinib, as well as many other vasorelaxant agents, seemed to require a pre-constriction to have a vasodilating effect in pulmonary vessels. The effect can be observed after pre-treatment with several different agents (ET-1, U46619, 5HT, L-NAME) that increase the vascular tone by affecting different signalling pathways (see 1.1.2.1). Most of these signalling pathways have also been found to be significantly more

active in PAH. For testing the potential of vasorelaxing agents in regard to PAH therapy, it is important to create a valid model of pulmonary vessels in PAH. In our trial we used ET-1, a selective ET_A receptor agonist. The expression of both the receptor and its ligand, ET-1, are increased in PAH animal models (see 1.1.2.1).

Imatinib was shown to decrease PAP and systemic arterial pressure (SAP) in rats after i.v. pre-treatment with L-NAME (a NOS-inhibitor) or U46619 (a TXA_2-receptor agonist) both of which increase the PAP. Without pre-treatment, however, there was only a small decrease of PAP (Pankey, Lasker, et al. 2013). Other trials showed that Imatinib relaxes rat PA rings pre-constricted with L-NAME, U46619 or 5-HT (Abe et al. 2011).

So far there is no evidence that imatinib directly inhibits the receptors or downstream signalling of any of the vasoconstricting agents mentioned above. Nor is any of the mentioned agents known to depend on PDGF signalling for their effects. Whether there is a common convergent mechanism that is activated by all of these vasoconstrictors and directly inhibited by imatinib remains to be investigated.

An alternative explanation for the effect of imatinib would be the presence of a constitutionally active tyrosine kinase that increases the baseline vascular tone and is inhibited by imatinib. However, this explanation leaves the question why the inhibition of aforementioned tyrosine kinase by imatinib should lead to significant vasodilation only after pre-constriction. One possible reason could be a biophysical enhancement of the basal activity of this tyrosine kinase through any vasoconstriction regardless of the biochemical cause. Biophysical effects on the vascular tone are an established concept: an increased sensitivity for endothelium dependent vasodilators in pre-constricted rat resistance arteries has been reported (Colton et al. 2012). However, further examination of eventual cross-linking between signalling of PDGF and the vasoconstrictors used for pre-constriction is necessary.

Since we only used ET-1 for pre-constriction in this trial, it could provide interesting insights to examine the vasorelaxant effect of imatinib on murine PCLS pre-treated with other of the abovementioned vasoconstricting agents.

One signalling pathway that at least U46619, 5-HT and ET-1 seem to converge on is the activation of RhoA and ROCK, which leads to reduced MLC phosphatase (MLCP)-activity and increased MLC phosphorylation and calcium sensitivity (Barman 2007; Homma et al. 2007). While recent trials showed that PDGF-BB-induced vasoconstriction of guinea pig PVs is ROCK-independent (Rieg et al. 2018), a possible inhibition of ROCK by imatinib has not been examined yet.

Clinical safety of Imatinib

Currently Imatinib promises to be a potent drug in the therapy of PAH. However, further studies will be necessary both concerning its beneficial effect and its safety. Cardiotoxicity and selective pulmonary vascular toxicity are being discussed as possible dangers of TKIs and recent data suggests severe side effects with increased mortality e.g. subdural hematoma, syncope and pleural effusion, under long-term treatment with Imatinib (Frost et al. 2015; Godinas et al. 2013). Further, single cases of reversible selective pulmonary vascular toxicity due to Dasatinib, an unspecific TKI, have been reported (Godinas et al. 2013). Further research will be necessary to assess the efficacy and safety of Imatinib and other TKIs as clinical drugs in PAH therapy in regard to specific patient groups with maximum benefit and minimal risk profile (e.g., subdural hematoma occurred predominantly in patients who were also taking anticoagulating medication). At the same time an interesting approach to targeted therapy is made with a new orally active pulmonary homing peptide that promotes homing of a drug to the pulmonary circulation without being linked to the drug (Toba et al. 2014). Thus, systemic side effects could be minimized. Furthermore, other TKIs may well be as effective on PAH as Imatinib and safer at the same time.

The mouse as an animal model for PH research

In PH research, the mouse is a frequently used animal model due to the options of inducing PH and creating knock-out and knock-down species. Recently there have been successful attempts to create a more human-like model of PH in mice by adding VEGFR-TKI to the hypoxia-treatment (Ciuclan et al. 2011; Van et al. 2014). While this might succeed in making the mouse an even better suited model for PH, several difficulties of this model arise for trials using PCLS. Due to their small size, murine lungs are more fragile than those of bigger animals, resulting in a higher risk of structures of interest being destroyed in the preparation process. The embedding of the lungs in agarose gel is a difficult step that has to be done quickly, nonetheless if the angle of the lung in the agarose cylinder is not correct, the chance of obtaining useful slices is reduced drastically. An extremely sharp blade is generally needed for the slicing, this is even more important for the slicing of mouse lungs, making it necessary to change the steel blades after every single lung in order to avoid ripping of the tissue. Even so, the delicacy of the tissue and the small volume of the pulmonary vessels increase the probability of a deformation during slicing that renders the vessels without visible lumen and the slices therefore useless. At the same time, the small lungs allow for fewer slices, reducing the number of suitable slices per lung. The PCLS themselves also are more fragile and the vessels smaller than those of bigger animals. Thus, during experiments external disturbances might have a greater influence on the measurements.

5.2 Limitations of this study

For this study, we used healthy mice with intact pulmonary vessels. Some of the molecular mechanisms we examined might be significantly altered in the lungs of mice with PH-related pathological remodelling of the pulmonary vessels. Further, the only murine pulmonary vessels available for PCLS experiments are central, large vessels due to the small size of the animals. The regulation of the pulmonary vascular tone occurs mainly in the smaller arterial and venous vessels. While the use of PCLS is a well-established method of examining pulmonary tissue in vitro, it would be desirable if our

results could be confirmed in additional trials using ventilated and perfused lungs or live animals.

6. Conclusion

In the past few years there have been several trials demonstrating a direct effect of PDGF, specifically PDGF-BB, and the TKI imatinib, on the pulmonary vascular tone. Some of these studies used the incubation of precision cut lung slices (PCLS), generated from human and guinea pig lungs, with the effector substances. The aim of those studies was to further the understanding the mechanisms of vascular tone dysregulation in PH as well as the possible use of imatinib as a novel targeted therapeutic drug for PH.

The mouse is an important model for PH research, and the PCLS method is already well established for this model, mostly for research on the airways. However, there has been no study yet to examine the effect of PDGF-BB or imatinib on the pulmonary vascular tone in the murine lung. Further, the molecular mechanisms involved in the regulation of the pulmonary vascular tone are as yet incompletely understood in any model. The main goal of this study is to determine whether there is a direct effect of PDGF-BB and imatinib on the pulmonary vascular tone that can be detected using the PCLS method. We also aimed to study the signalling on which such an effect might depend.

In this study, we found a vasorelaxing effect of imatinib in pre-constricted murine PAs and PVs. We are also able to demonstrate that PDGF-BB constricts murine PAs and PVs. In murine PAs, we showed that the contractile effect of PDGF-BB is prevented by imatinib, and that it depends on PI3K-α/γ and on LTCCs, but not on the regulation of NO synthesis. Our results were conclusive and often in line with preliminary findings in different animal models (Rieg et al. 2018), confirming that the PCLS-method is suitable for research on the murine lung vessels if certain limitations are considered (see above). Future research on the underlying mechanisms may provide further insights into the pathogenesis of PH and lead to the discovery of new molecular targets for specific therapies, among them possibly PDGFR-β, PI3K and Rho-kinase. The therapy of PH is still rapidly developing, and ongoing research is likely to create more effective drugs for the treatment of this lethal disease.

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Anhang

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Erklärung § 5 Abs. 1 zur Datenaufbewahrung

Hiermit erkläre ich, Christian Cranen, dass die dieser Dissertation zugrunde liegenden Originaldaten im Institut für Pharmakologie und Toxikologie des Universitätsklinikums Aachen, Wendlingweg 2, 52074 Aachen, hinterlegt sind.

Christian Cranen (Doktorand)

Erklärung über den Eigenanteil

Eidesstattliche Erklärung gemäß § 5 Abs. (1) und § 11 Abs. (3) 12. der Promotionsordnung

Hiermit erkläre ich, Christian Cranen**,** an Eides statt, dass ich folgende in der von mir selbstständig erstellten Dissertation „The pulmonary vascular effect of PDGF and Imatinib in murine precision cut lung slices“ dargestellten Ergebnisse erhoben habe:

Bei der Durchführung der Arbeit hatte ich folgende Hilfestellungen, die in der Danksagung angegeben sind:

	C. Cranen	Fr. PD Dr. med. Annette Rieg	Fr. N. Ruske	Fr. Prof. Dr. med. R. Knüchel-Clarke	Prof. Dr. rer. nat. C. Martin	Univ.-Prof. Dr. rer. nat. St. Uhlig	Summe (%)
Studienüberwachung		80			20		100
Studiendesign/Konzeption	20	40			40		100
Pflege d. Mäuse	80		20				100
Herstellung d. PCLS	100						100
Bereitstellung der Materialien						100	100
Durchführung d. Experimente	100						100
Statistische Auswertung	70	30					100
Interpretation d. Datenauswertung	60	40					100
Verfassen d. Dissertation	100						100
Korrektur d. Dissertation		80			20		100
Beurteilung d. Histologie				100			100

_____

Christian Cranen (Doktorand)

Als Doktorvater bzw. als Betreuerin der obigen Dissertation bestätigen wir die Angaben von Christian Cranen

_____ _____

Prof. Dr. rer.nat. Christian Martin PD Dr.med. Annette Rieg