EFFECTS OF C-REACTIVE PROTEIN ON HUMAN PULMONARY VASCULAR CELLS IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION

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Running title: CRP and pulmonary vascular cells in CTEPH

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ABSTRACT

CTEPH is characterized by proximal pulmonary vascular obstruction by thrombo-fibrotic material of which origin is not elucidated. Enhanced inflammation could contribute to persistent obstruction by impairing pulmonary vascular cell function in CTEPH. We investigated C-reactive protein (CRP) effects on pulmonary vascular cell function in vitro.

Primary cultures of proximal pulmonary endothelial (EC) and smooth muscle (SMC) cells from CTEPH and non-thromboembolic pulmonary hypertension (PH) patients were established. Recombinant CRP effects on mitogenic activity, adhesion capacity, endothelin-1 and von Willebrand factor (vWF) secretion and adhesion molecule ICAM-1 and VCAM-1 expression were investigated in EC and/or SMC. Expression of CRP receptor LOX-1 was evaluated in proximal pulmonary arterial tissue and cells by Western blotting and immunofluorescence.

CRP increased CTEPH-SMC proliferation by 250%. CRP increased adhesion capacity, endothelin-1 and vWF secretion by CTEPH-EC by 37%, 129% and 694%, respectively. CRP-induced adhesion of CTEPH-EC to monocytes was mediated by ICAM-1. CRP had no effect on cells from non-thromboembolic PH patients likely because of overexpression of LOX-1 in CTEPH. Local expression of CRP was detected in EC and SMC within pulmonary arterial tissue.

CRP may contribute to persistent obstruction of proximal pulmonary arteries in CTEPH by promoting vascular remodeling, endothelial dysfunction and in situ thrombosis.

Keywords: C-reactive protein, Chronic Thromboembolic Pulmonary Hypertension, Endothelial and Smooth Muscle Cells, Inflammation
INTRODUCTION

Chronic thromboembolic pulmonary hypertension (CTEPH) is one of the main causes of severe pulmonary hypertension. CTEPH is characterized by intraluminal thrombus organization and fibrous stenosis or complete obliteration of proximal pulmonary arteries. The consequence is an increased pulmonary vascular resistance resulting in pulmonary hypertension and progressive right heart failure. Pulmonary embolism, either as single or recurrent episodes, is thought to be the initiating event followed by progressive pulmonary vascular remodelling. CTEPH mainly differs from pulmonary arterial hypertension (PAH) by the proximal location of pulmonary artery obliteration [1].

To date, the physiopathology of CTEPH remains poorly understood. Proximal lesions share similarities with atherosclerotic plaques, including media thickening and neointima formation [2]. Besides evidence of dysregulated thrombosis and/or thrombolysis, an elevated prevalence of inflammatory diseases has been observed in CTEPH patients [3]. Accordingly, plasma levels of tumour necrosis factor α (TNFα) are elevated in CTEPH [4] and elevated pulmonary vascular resistance is correlated with increased expression of CCL2 chemokine monocyte chemoattractant protein-1 in plasma and large pulmonary arteries of CTEPH patients [5]. Furthermore, bacterial staphylococcal infection has been shown to prevent the resolution of inferior vena cava thrombi in rodents and is observed in most patients with ventriculo-atrial shunt or pace maker related CTEPH [6].

We have recently shown that CTEPH patients display elevated circulating C-reactive protein (CRP) levels [7]. CRP, a marker of acute inflammation and tissue damage, is an independent risk factor for cardiovascular and systemic diseases [8]. Besides its commonly accepted role of bystander in various inflammatory diseases, CRP also plays an active role in atherogenesis [9,10]. Indeed CRP locally induces the production of vasoconstrictive, thrombotic, proliferative and inflammatory molecules in systemic arteries [10]. These molecules could also be involved in the pathogenesis of CTEPH.

Endothelin-1 (ET-1) and von Willebrand factor (vWF) are important players in the pathophysiology of pulmonary hypertension [11]. ET-1 and vWF plasma levels are elevated in patients with CTEPH and correlate with clinical parameters [12-14]. ET-1 is secreted by CRP-stimulated venous endothelial cells [15].
vWF is released from its endothelial storage compartment, the Weibel-Palade bodies, when the endothelium is damaged and is consequently considered as a marker of endothelial dysfunction [16]. vWF stabilizes factor VIII, a key player of the coagulation cascade and a risk factor for CTEPH, and therefore contributes to thrombogenesis [17].

The mechanisms resulting in the persistence of obstructing fibro-thrombotic material in CTEPH require being unravelled. We consequently hypothesized that enhanced inflammation may be involved in that process. Our objective was to investigate if CRP could be more than a bystander in CTEPH, by investigating its effect on vascular remodelling, endothelial dysfunction and thrombosis in proximal pulmonary vessels of CTEPH patients, using primary cultured pulmonary artery smooth muscle (SMC) and endothelial cells (EC), and pulmonary arterial tissue.
MATERIALS & METHODS

Study population

Proximal pulmonary vascular tissue was obtained from i) CTEPH patients who underwent a pulmonary endarterectomy (PEA) and ii) patients with other causes of pulmonary hypertension (PAH or due to lung diseases), assigned as non-thromboembolic pulmonary hypertension (PH), who underwent a lung transplantation. Demographics were recorded. Pulmonary hemodynamic parameters were collected in anesthetised patients in the operation room before starting lung transplantation or PEA. Plasma CRP levels were measured in the University Hospital routine lab as previously described [7] within 24 hours before PEA and lung transplantation. The study protocol was approved by the Institutional Ethics Committee of the University Hospital of Leuven and participants gave written informed consent.

Tissue collection

At the time of lung transplantation or PEA, a 2-cm piece of proximal pulmonary artery, free from thrombotic material, was stored in cell culture media containing antibiotics and antifungal agents at 4°C. Two pieces were quickly frozen in liquid nitrogen and stored at -80°C until protein extraction. Another piece was fixed in 4% paraformaldehyde and cryoembedded.

Cell culture

Proximal EC were obtained by collagenase type II (1 mg.mL⁻¹; LifeTechnologies) digestion in HBSS for 20 min at 37°C. EC were seeded on gelatin-coated (Sigma-Aldrich, Bornem, Belgium) cell culture flasks. Immunomagnetic purification of EC was performed using anti-platelet endothelial cell adhesion molecule-1, also known as CD31, monoclonal antibody–labeled beads, according to the manufacturer’s instructions (Miltenyi Biotec, Utrecht, The Netherlands). EC morphology was confirmed by more than 95% cells positive for CD31, vWF and Dil-Ac–LDL staining. Proximal SMC were isolated using an explant-outgrowth method. SMC were seeded on fibronectin-coated (R&D Systems) cell culture flasks. To determine the phenotypic characteristics of cultured SMC, cells were assessed for expression of muscle-specific contractile and cytoskeletal proteins including smooth muscle α-actin, desmin, and smooth muscle myosin heavy chain. EC and SMC were used between passages 3 and 6.
EC were cultured in M199 medium (Life Technologies, Gent, Belgium), supplemented with 20% fetal bovine serum (FBS) 100 U.mL\(^{-1}\) penicillin, 100 µg.mL\(^{-1}\) streptomycin, 1.25 µg.mL\(^{-1}\) fungizone (Life Technologies), 10 U.mL\(^{-1}\) heparin (Aventis, Brussels, Belgium) and 5 ng.mL\(^{-1}\) α-FGF (R&D Systems, Abington, UK). SMC were grown in DMEM medium (Life Technologies) supplemented with 10% FBS, penicillin, streptomycin and fungizone. U937 cells were cultured in RPMI (Life Technologies) containing 10% FBS, penicillin, streptomycin and fungizone.

**Cell proliferation**

Subconfluent SMC and EC were starved for 24h in DMEM and M199 supplemented with 0.2% FBS, respectively. Mitogenic activity of EC and SMC was measured in the presence of increasing concentrations of CRP (endotoxin-free, highly purified human recombinant, R&D Systems) plus 0.5 µCi.mL\(^{-1}\) of \(^{3}H\)-thymidine (74GBq.mmol\(^{-1}\); GE Healthcare, Buckinghamshire, UK) for 48h. Trichloroacetic acid-insoluble material was solubilised in 0.2 N NaOH and radioactivity incorporation was quantified in a β-scintillation liquid counter (LS5000CE, Beckman, Fullerton, CA).

**Cell adhesion assay**

Confluent EC were starved for 6h and stimulated with human recombinant CRP (10 ng.µL\(^{-1}\)) for 3h. Conditioned medium was collected and stored at -20°C until further use. Human monocytic U937 cells in suspension were radio-labelled with 1µCi \(^{3}H\)-thymidine/10\(^6\) cells for 48h and added (5x10\(^5\)/well) to the endothelial cell monolayer for 3h at 37°C. Non-adherent cells were washed out. Radioactivity incorporated into attached monocytes to EC monolayer was quantified as described above.

**Adhesion molecule expression at cellular surface**

Cells were incubated with CRP (10 ng.µL\(^{-1}\)) for 3h at 37°C and fixed with paraformaldehyde. Adhesion molecule expression was performed by using antibodies against ICAM-1 or VCAM-1 (R&D Systems) Fluorescence labelling was obtained using the secondary antibodies Alexa594 goat anti-mouse (Life Technologies). Staining with 4’,6-diamidino-2-phenylindole (DAPI) was used to visualize nuclei.

**Immunofluorescence on tissue**

Co-localization of LOX-1 and CRP with EC or SMC was performed on 10-µm cryosections using a mixture of two primary antibodies against CD31 (EC; Dako, Heverlee, Belgium) or α-SMA (SMC, Dako) and LOX-1
(Abcam, Cambridge, UK) or CRP (Abcam). Fluorescence labelling was obtained using the secondary antibodies Alexa488 goat anti-mouse, Alexa594 goat anti-rabbit or Alexa594 goat anti-sheep (Life Technologies). Staining with DAPI was used to visualize nuclei.

**Western blotting**

Expression of lectin-like oxidized low-density lipoprotein (oxLDL) receptor-1 (LOX-1), a potential receptor of CRP, in pulmonary vascular tissue and in cultured endothelial cells was performed by Western blotting using specific primary antibodies against LOX-1 (Abcam, Cambridge, UK). β-actin was used as an internal control. Horseradish peroxidase-conjugated donkey anti-rabbit IgG for anti-LOX-1 and anti-mouse IgG (Kackson, Suffolk, UK) for anti-β-actin were used as secondary antibodies. Peroxidase staining was revealed with a chemiluminescence kit (GE Healthcare) and performed with films exposed at room temperature. Protein expression was quantified using the Photoprint imaging system and the BIO-1D software (Vilber Lourmat, Marne-la-Vallée, France).

**Endothelin-1 measurement**

ET-1 levels were measured in the collected conditioned medium of EC using an ELISA kit following the manufacturer’s instructions (R&D Systems).

**Von Willebrand factor measurement**

vWF antigen concentrations were determined in conditioned medium of CRP-stimulated EC collected after wash-out of 4, 18, 24 and 48h by enzyme-linked immunoabsorbent assay (ELISA) with rabbit polyclonal anti-human VWF (Dako) as described elsewhere [18].

**Statistical analysis**

Database management and statistical analyses were performed using SAS Enterprise Guide 4.1 (SAS Inc., Cary, North Carolina) and GraphPad Prism 4.01 (GraphPad Software Inc., La Jolla, California). Data were expressed as mean±SEM in the figures. Differences between the 2 groups were analyzed using one or two-way ANOVA test followed by post-hoc tests or Student t-test. A value of p<0.05 was considered statistically significant. All p values were for 2-sided tests.

A detailed method section can be found in the online supplement.
RESULTS

Patient characteristics

We obtained proximal pulmonary arterial tissue from 26 CTEPH patients and 15 patients with non-thromboembolic PH (6 PAH, 9 PH due to lung diseases including COPD, idiopathic pulmonary fibrosis and sarcoidosis). Among PAH patients, 3 had idiopathic PAH, 2 PAH associated with congenital heart disease and one PAH associated to anorexigen intake. Except one patient with a very complex form of congenital cardiopathy, all PAH patients were under parenteral prostanoid therapy, together with Bosentan and/or Sildenafil, at the moment of the transplantation. Demographics, pulmonary hemodynamic and circulating CRP levels were likely similar in both groups (see Table).

Effects of recombinant CRP on pulmonary artery cells in vitro

Increasing CRP concentrations significantly increased CTEPH-SMC mitogenic activity by 250%, but had no effect on PH-SMC (p=0.003; Figure 1A). CRP had no effect on EC mitogenic activity (Figure 1B). CRP increased human monocytic cells (U937) cell adhesion to CTEPH-EC by 37% (p<0.005) whereas it had no effect on adhesion capacity of PH-EC (Figure 2). CRP induced the expression of ICAM-1 exclusively at CTEPH-EC surface (Figure 3), whereas it has no effect on VCAM-1 expression (data not shown). In addition, we did not observe any significant difference regarding response to CRP between cells isolated from PAH patients and cells isolated from patients with PH due to lung diseases (data not shown).

CRP receptors expression

CRP Fcy receptor CD64 mRNA was equally expressed in PH and CTEPH pulmonary vascular tissue, whereas CD32 mRNA expression was significantly up-regulated in CTEPH (data not shown). However, CD32 protein expression was similar in both CTEPH and PH (data not shown). By contrast, LOX-1 protein expression was significantly up-regulated in CTEPH pulmonary artery tissue and cultured EC (p<0.005 and p<0.05, Figure 4A & 4B). LOX-1 co-localized with CD31 positive CTEPH-EC, but not with CD31 positive PH-EC (Figure 4C & 4D). Since CRP had only significant effect on pulmonary cells from CTEPH patients in vitro, likely through an enhanced expression of LOX-1 receptor, further experiments were performed on cells or tissue from CTEPH patients.
Effects of CRP on the secretion of vasoconstrictors and thrombotic factors

CRP increased significantly the secretion of ET-1 by 129% and the secretion of vWF by 694% by CTEPH-EC (Figure 5). CRP did not induce any release of factor VIII, nor tissue factor (data not shown).

Local CRP production in pulmonary arterial tissue and cells

CRP mRNA and protein expression was detected in pulmonary vascular tissue of patients CTEPH (data not shown). CRP expression was highly detected by immunofluorescence both in SMC and EC from pulmonary artery tissue of CTEPH patients (Figure 6).
DISCUSSION

This paper shows that the inflammation marker CRP is able to enhance SMC mitogenic activity, inflammatory cell adhesion to EC, secretion of ET-1 and vWF by EC from CTEPH patients. This suggests that CRP could play a role in the pathophysiology of CTEPH by contributing to the persistence of fibro-thrombotic material obstructing proximal pulmonary arteries. To our knowledge, this is the first study evidencing a potential contribution of CRP to pulmonary artery cell dysfunction and vascular wall remodelling in CTEPH.

The physiopathology of CTEPH remains incompletely understood. In most cases it is associated with a history of acute venous thromboembolism [19], but wherefore thrombi do not resolve after an acute event in a low percentage of patients is unclear. The current state of knowledge is based on a triad of enhanced thrombosis [12,20], disturbed thrombolysis [21-23] and inflammation [3]. These mechanisms were all approached in the current experiments regarding CRP effects.

Bernard et al [24] by reviewing 200 PEA specimens of fibrotic organized thromboembolic material that adheres to the vessel wall observed cellular proliferation in the wall of recanalizing lumens and in the stroma between the lumens. Myxoid changes were prominent in those areas, as well as inflammation, and the authors stated that unknown stimuli for cellular proliferation and inflammatory infiltration might be involved in the pathogenesis of CTEPH. Sacks et al [25] extensively reviewed the mechanisms that could be responsible for proximal vessel remodelling in CTEPH. However, experimental data regarding the physiopathology of CTEPH are extremely limited.

CRP is an independent biomarker of various inflammatory diseases [9]. We have recently found elevated circulating CRP levels in CTEPH patients and CRP was significantly decreased 12 months after PEA [7]. Besides its known role of bystander and marker of clinical risk in inflammatory diseases, CRP may also be an active player in the pathophysiology of the vascular wall, as already demonstrated in atherosclerosis [26,27].

In the current study, proximal pulmonary vascular cells isolated from CTEPH patients have been compared to proximal pulmonary vascular cells isolated from patients with non-thromboembolic PH
because both pulmonary vasculature are exposed to similar high pressure (see Table). Distal pulmonary vascular cells isolated from PAH patients have been shown to display enhanced mitogenic activity and ET-1 secretion \textit{in vitro} compared to control [28]. Nevertheless, proximal pulmonary vascular cells isolated from PAH patients or patients with PH due to lung diseases harbour a low proliferation rate even in the presence of high concentration of FBS (data not shown) and do not secrete ET-1, \textit{in vitro}. This may suggest that proximal pulmonary vascular cells may harbour different features potentially attributable to the heterogeneity of pulmonary vascular cells along the pulmonary vascular tree [29].

\textit{In vitro effects of CRP on pulmonary vascular cells}

The vascular material collected during PEA consists of the neointima and few elastic lamellae from the tunica media of pulmonary arteries and, if present, recent and/or old thrombotic endoluminal material. For the cell culture experiments, fresh and organized thrombotic material was discarded. Neointima formation seems to be an important component of proximal pulmonary artery remodelling in CTEPH, and the CRP induced CTEPH-SMC proliferation evidenced in our experiments could be part of the explanation for this observation (figure 7). The ET-1 pathway could also be involved in this remodelling (figure 7). We observed that CRP addition to our CTEPH-EC induced ET-1 secretion. Others have already shown elevated circulating ET-1 levels in CTEPH patients, together with a correlation with clinical parameters [13]. Addition of CRP also induced ET-1 release in human saphenous vein EC [15] and adeno-associated virus-mediated CRP gene overexpression caused an increase in ET-1 circulating levels and local aortic expression of ET-1 mRNA in rats [30]. ET-1 also enhanced the proatherosclerotic effects of CRP in the systemic circulation [31].

In the present study, we have observed that CRP-induced enhanced adhesion capacity of CTEPH-EC to the monocytic human cell line U937 could be attributed to CRP-induced ICAM-1, but not to VCAM-1 expression at the EC surface. By contrast, in human umbilical vein EC, CRP induces VCAM-1, ICAM-1 and E selectin expression at the cell surface [32]. This could thereby explain adhesion and further migration into the vessel wall of inflammatory cells [24] (Figure 7). Taken together, the results of CRP effects on CTEPH-EC and CTEPH-SMC suggest a potential role of CRP in endothelium activation and in remodelling of the pulmonary vascular wall in CTEPH. CRP-induced effects on remodelling could be either mediated by EC through ET-1 release or occur directly, as evidenced by CRP-induced CTEPH-SMC mitogenic activity.
Our observation that CRP can induce the secretion of vWF by CTEPH-EC suggests that CRP could contribute to thrombogenesis in CTEPH and consequently to the persistence of pulmonary vessel obstruction (Figure 7). Infusion of CRP in human volunteers results in marked elevations of vWF, prothrombin, D-dimers and plasminogen activator inhibitor type 1 (PAI-1) [33]. CRP also contributes to vascular thrombosis in the systemic circulation by decreasing NO bioavailability, inhibiting PGI2 activity, and inducing tissue factor and PAI-1 release [8]. Plasma levels of vWF and of clotting factor VIII have been shown to be elevated in CTEPH patients compared to controls and PAH patients [12], and differences in the expression of PAI-1 have been observed inside pulmonary thrombi [34].

The specific effects of CRP on proximal pulmonary vascular cells from CTEPH patients could likely be attributed to a differential expression in LOX-1 receptor. In the systemic circulation, CRP mediates its biological effects on endothelial cells mainly via binding to the Fcy receptors CD32 and CD64 [35]. However, we did not find any significant differences in the expression on CD32 and CD64 in pulmonary vascular tissue from CTEPH and non-thromboembolic PH patients. It has recently been shown that CRP is also a ligand of LOX-1, the primary endothelial receptor for oxLDL [36]. Moreover oxLDL induced ICAM-1 and VCAM-1 mRNA expression [36] and ET-1 secretion by human umbilical vein EC [37].

Regarding a potential interference of the anti-inflammatory effects of PAH targeted therapy with the current results, we observed that circulating CRP levels measured in PAH patients before starting prostanoid therapy and at the time of lung transplantation were unchanged [4.4 (95%CI: 1.1-17.2) vs. 11.4 (95%CI/ 5.4-23.9) mg.L⁻¹, p=0.11], as previously shown on a larger number of patients [7]. Moreover, the patient with PAH associated with congenital heart disease, who did not receive any prostanoid therapy before transplantation, displayed a low LOX-1 expression level similar to the other PAH prostanoid-treated PAH patients. By contrast, 2 CTEPH patients received epoprostenol for one to 6 months prior to PEA. Circulating CRP levels dropped in these patients. However, each of them showed either an increased adhesion of monocytes to EC in response to CRP or a high LOX-1 expression level in the proximal pulmonary arteries. Considering the very short half-life of prostanoid molecules such epoprostenol (6 min) or treprostinil (5 h), the chance that an effect of the drug on cells could be maintained in culture for about one
month remains very poor. Taken together, all the above observations suggest that prostanoid therapy could hardly interfere with CRP effects on cells \textit{in vitro}.

\textbf{Pulmonary vascular production of CRP}

In agreement with previous findings reporting that vascular SMC are the major source of CRP local production in systemic injured or atherosclerotic arteries [38] and that vascular EC may also produce CRP [39], this is the first study reporting a local production of CRP by EC and SMC in CTEPH proximal pulmonary arterial tissue. This locally produced CRP might contribute to endothelial dysfunction, vascular remodelling and \textit{in situ} thrombosis in CTEPH (Figure 7).

\textbf{Relevance of the study}

Since the pathophysiology of CTEPH remains poorly understood, an original and unexplored approach was to establish primary cultures of EC and SMC derived from CTEPH patients. Our study supports the concept that besides being a surrogate [7], circulating CRP and locally produced CRP could actively contribute to the pathophysiology of CTEPH. Recently, a simple non-invasive diagnostic algorithm, including CRP, has been developed for ruling out CTEPH in patients after acute pulmonary embolism [40].

\textbf{Conclusion}

Using primary cultures of EC and SMC derived from CTEPH patients, we have evidenced a potential CRP-driven dysfunction of pulmonary vascular cells in CTEPH. In addition to a direct effect of circulating CRP on endothelial cells, locally produced CRP by pulmonary vascular cells could also contribute to vascular remodelling, endothelial dysfunction and \textit{in situ} thrombosis in CTEPH. Consequently, our results suggest that the inflammation marker, CRP, could play a role in the pathophysiology of CTEPH by contributing to persistence of obstructing material in proximal pulmonary arteries.
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REFERENCES


FIGURE LEGENDS

Figure 1. Effects of CRP on pulmonary vascular cell proliferation. Mitogenic activity of proximal SMC (A) isolated from patients with non-thromboembolic PH (n=3; patients 112, 114, 123) and CTEPH patients (n=3). ANOVA, p = 0.003, *p=0.02 vs. PH. Mitogenic activity of proximal EC (B) isolated from patients with non-thromboembolic PH (n=3) and CTEPH patients (n=3). For each patient, 1 to 2 independent experiments were performed in triplicate.

Figure 2. Effect of CRP on adhesion capacity of EC. Adhesion capacity of proximal EC isolated from patients with non-thromboembolic PH (n=4) and CTEPH patients (n=4) to monocytic cell line U937 in the absence (control) and in the presence of 10 µg.mL⁻¹ CRP. ANOVA, p=0.002, *p<0.005 vs. PH-EC-CRP₁₀. For each patient 1 to 3 independent experiments were performed in triplicate.
Figure 3. CRP effects on cell adhesion molecules expression in EC. Proximal EC derived from patients with non-thromboembolic PH (A, B, C) and CTEPH patients (D, E, F) were either not stimulated (A, D) stimulated by either 10 ng.mL$^{-1}$ TNF-α (B, E) or 10 μg.mL$^{-1}$ CRP (C, F) for 3 h. Cells were further stained with an antibody raised against ICAM-1 (red). Nuclei were counterstained using DAPI (blue).
Figure 4. LOX-1 expression and localization in pulmonary vascular cells and tissue. LOX-1 protein expression was measured in the proximal pulmonary arteries (A) and primary cultured EC (B) of patients with non-thromboembolic PH (n=10 for PA; n=3 for EC) and patients with CTEPH (n=10 for PA; n=4 for EC); unpaired t-test, *p=0.03; **p=0.001. Sections of pulmonary vascular tissue from patients with non-thromboembolic PH (C) and CTEPH patients (D) were stained with antibodies against LOX-1 (red) and CD31 (green). The 2 stainings were merged (yellow). Nuclei were counterstained using DAPI (blue).
Figure 5. Effects of CRP on endothelial function. (A) ET-1 secretion by proximal EC isolated from CTEPH patients (n=4) in the absence (control) and in the presence of CRP (10 µg.mL⁻¹). For each patient 1 to 3 independent experiments were performed in triplicate and measurements in duplicate. *p=0.001. (B) vWF secretion by CTEPH-EC (n=6) stimulated by CRP. Conditioned medium was collected after 4, 18, 24 and 48h. ANOVA, p<0.0001, *p<0.05, **p<0.001 vs. control.
Figure 6. Localization of CRP in pulmonary vascular tissue sections. Sections of pulmonary vascular tissue from CTEPH patients were stained with antibodies against CRP (red) and α-SMA (green; A) or CD31 (green; B). The 2 stainings were merged (yellow). Nuclei were counterstained using DAPI (blue).
Figure 7. Direct and indirect effects of CRP on endothelial function and vascular remodeling in CTEPH.

Increased circulating CRP levels in CTEPH contribute to activate the endothelium through the receptor LOX-1, resulting in i) expression of ICAM-1 at the endothelium surface, ii) attraction of circulating monocytes, iii) secretion of vWF and iv) secretion of ET-1, which in turn may induce SMC proliferation. Within the vascular wall, SMC proliferation may be induced by CRP produced by EC or by SMC themselves.
Figure 7

Circulating CRP

CRP

ET-1

CRP

Blood

vWF

monocyte

SCAM-1

EC

SMC
Table. Patient characteristics

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mPAP, mean pulmonary arterial pressure (measured by right heart catheterization); RAP, right atrial pressure; TPR, total pulmonary vascular resistance; CO, cardiac output; CRP, C-reactive protein. CRP data were expressed as geometric mean (95% confidence interval). Circulating CRP and hemodynamic parameters have been measured at the time of PEA or transplantation.