Circulating Microparticles from Pulmonary Hypertensive Rats Induce Endothelial Dysfunction

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Rationale: Pulmonary arterial hypertension (PAH) is a severe disease characterized by an increase of pulmonary vascular resistance, which is accompanied by functional and structural changes in pulmonary arteries. Microparticles (MPs) have been described as biological vector of endothelial dysfunction in other pathologies.

Objectives: The purpose of this work was to characterize circulating MPs during hypoxic PAH and to study their effects on endothelial function.

Methods: Male Wistar rats were exposed or not to chronic hypoxia, and normoxic or hypoxic MPs from blood were characterized by flow cytometry. Endothelial cells (ECs) from rat aorta or pulmonary arteries were incubated with MPs, and then expression and phosphorylation of enzymes involved in nitric oxide (NO) and reactive oxygen species productions were analyzed. Hypoxic MPs were injected into rats, and endothelium-dependent relaxation was assessed.

Measurements and Main Results: Circulating levels of MPs from hypoxic rats were twofold higher than those present in normoxic rats. In vitro treatment of ECs with hypoxic MPs reduced NO production in aortas and pulmonary arteries by enhancing phosphorylation of endothelial NO synthase at the inhibitory site. Hypoxic MPs increased oxidative stress only in pulmonary ECs via xanthine oxidase and mitochondrial implication. In vivo injection of hypoxic MPs into rat impaired endothelium-dependent relaxation both in aorta and pulmonary arteries.

Conclusions: These data provide evidence that hypoxic circulating MPs induce endothelial dysfunction in rat aorta and pulmonary arteries by decreasing NO production. Moreover, MPs display tissue specificity with respect to increased oxidative stress, which occurs only in pulmonary ECs.

Keywords: pulmonary hypertension; microparticles; oxidative stress; endothelial NO synthase

Pulmonary arterial hypertension (PAH) is a rare and severe disease characterized by an increase in pulmonary vascular resistance leading to right ventricle overload and, ultimately, death (1). From a pathophysiological point of view, it is well established that in PAH the main abnormality is an endothelial dysfunction (2), which correlates with a decrease in vasodilator factor release, such as nitric oxide (NO) (3) or prostacyclin, as well as an increase in vasoconstrictor production, endothelin-1, which is able to stimulate cell proliferation. NO generated from endothelial NO synthase (eNOS) plays a key role in the vascular homeostasis. Besides its vasodilator properties on smooth muscle cells, NO inhibits proliferation and migration of these cells and can regulate vascular remodeling. In addition, several studies have shown that oxidative stress initiated by the reactive oxygen species (ROS) plays a determinant role in the reduced effect of endothelial NO and may take place in vasculature from patients with PAH or in animal models developing PAH (4–6). Among the ROS, superoxide anions (O2•−) are known to reduce biological activity of NO and generate deleterious metabolites such as peroxynitrite (ONOO−) (7).

Recently, two groups reported a positive correlation between plasma concentration of circulating microparticles (MPs) and the severity of PAH (8, 9). MPs are vesicles with procoagulant and proinflammatory properties shed from the blebbing plasma membrane of various cells types, like circulating cells (such as platelets, erythrocytes, T and B cells, and monocytes) or cells from the vascular wall (endothelial and smooth muscle cells), during activation by agonists, shear stress, or apoptosis. MPs are present in blood from healthy individuals and many studies have reported elevated levels of circulating MPs under many pathological states, such as cardiovascular diseases (for review see 10), strengthening the notion that MPs may play a role in the initiation or maintenance of several components of these diseases. Indeed, MPs can be considered as vectors of biological messages, such as induction of vascular and endothelial dysfunction, the main factor of PAH pathophysiology.

Contradictory data concerning the origin of MPs from patients with PAH have also been described. Although Bakouba and colleagues (8) have described that these patients display elevated levels of procoagulant MPs, Amabile and colleagues (9) have shown that they are mainly from endothelial and leukocyte origins. However, no evidence of the potential effects of MPs on molecular mechanisms implicated in the

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject
Although several studies have shown that circulating microparticles (MPs) are elevated during pulmonary arterial hypertension (PAH), the origin and the role played by these MPs in the regulation of endothelial function are not known.

What This Study Adds to the Field
Increased circulating MPs from hypoxic rats induce endothelial dysfunction through both the decrease in nitric oxide production and an increase in oxidative stress in pulmonary endothelial cells.

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pathophysiology of PAH have been provided. Therefore, the present study investigates the effects of MPs from hypoxic rats on endothelial function. Because endothelial function of both systemic (11, 12) and pulmonary arteries (6) can also be affected by hypoxia, the present study assessed the effects and the mechanisms by which hypoxic MPs affect NO and ROS in endothelial cells (ECs) from aorta and from pulmonary arteries from the rat.

METHODS

Animals
All animal studies were performed using approved institutional protocols. Male Wistar rats (aged 8–10 wk, 250–300 g) were separate into two groups. Control or normoxic rats were housed in ambient room air, whereas hypoxic rats were exposed to chronic hypoxia for 3 weeks in a controlled hypobaric chamber. PAH was assessed by measuring the mean pulmonary artery pressure (15.6 ± 0.81 vs. 30.4 ± 1.02 mm Hg in normoxic vs. hypoxic rats, P < 0.05) and the ratio of right ventricle to left ventricle plus septum weight (0.26 ± 0.04 vs. 0.51 ± 0.01 in normoxic vs. hypoxic rats, P < 0.05). Normoxic or hypoxic MPs were extracted from rat blood.

MP Isolation
Blood from normoxic or hypoxic rats was collected in sodium citrate tubes after a cardiac puncture. Samples were centrifuged for 3 minutes at 1,900 × g, and then plasma was centrifuged for 4 minutes at 5,000 × g to obtain platelet-free plasma (PFP). PFP (100 μl) was frozen and stored at −80°C until subsequent use. As previously described (13), remaining PFP was subjected to three series of centrifugations at 15,000 × g for 30 minutes to eliminate plasma and to pellet MPs, and supernatant was replaced by 0.9% saline salt solution. Finally, MP pellets were stored at 4°C until subsequent use. Washing medium from the last supernatant was used as control (vehicle).

Characterization of MP Phenotype
Membrane MP subpopulations were discriminated in PFP according to the expression of membrane-specific antigens. Numeration of platelet, endothelial, leukocyte, and erythrocyte MPs was performed using anti-CD61, anti-CD54, anti-CD45 (BioLegend, San Diego, CA), and anti-Erythrocyte cell (BD Biosciences, San Jose, CA) labeling, respectively.

Cell Culture
Primary ECs were isolated from rat aortic and pulmonary arteries. The extraction method was adapted from the protocol of Kobayashi and colleagues (14). Cells were treated for 24 hours in the absence or presence of normoxic or hypoxic MPs at the circulating levels of MPs detected in the plasma of normoxic or hypoxic rats, as previously described for other pathologies (15–17).

NO and O$_2^-$ Determinations by Electron Paramagnetic Resonance
Detection of NO and O$_2^-$ productions were performed as previously described (15–17).

Xanthine Oxidase Activity
Determination of xanthine oxidase activity was performed using Xanthine Oxidase Assay Kit (BioVision Research Products, Mountain View, CA) according to the manufacturer’s instructions.

Western Blotting
Blots were performed as previously described (18).

Vascular Reactivity
Rats were treated in vivo by tail vein injection of hypoxic MPs at the circulating level detected in the plasma of hypoxic rats or the same volume of control. PAH was measured 24 hours after hypoxic MP injection. In another set of experiments, aortic rings and extrapulmonary arteries were isolated 24 hours after MP injection and mounted on

Figure 1. Circulating microparticle (MP) levels in hypoxic rats compared with normoxic rats. Histograms show (A) total circulating MP levels, and (B) platelet- (CD61$^+$), (C) erythrocyte- (erythroid), (D) leukocyte- (CD45$^+$), and (E) endothelium-derived (CD54$^+$) MPs from normoxic rats (NxMPs, n = 15) and hypoxic rats (HxMPs, n = 22). Results are expressed as events/μl of plasma and given as mean ± SEM. *P < 0.1; **P < 0.01, and ***P < 0.001.
Data Analysis

Data are expressed as mean ± SEM, and n represents the number of experiments performed with ECs or the number of rats for vascular reactivity. pD$_2$ = −log EC50, EC50 being the molar concentration of the agonist that produces 50% of the maximal effect. Statistical analyses were performed by two-way analysis of variance, and non-parametric Mann-Whitney U tests or analysis of variance for repeated measures and subsequent Bonferroni post hoc test. P values less than 0.05 were considered to be statistically significant.

See the online supplement for additional details on the methods for making these measurements.

RESULTS

Circulating Levels of MPs and Their Cellular Origins

The total number of circulating MPs was significantly increased in hypoxic rats compared with normoxic rats (Figure 1A). Phenotypical characterization of the cellular origin of MPs showed a significant increase in the circulating levels of platelet (CD61$^+$)- and erythrocyte-derived MPs in hypoxic rats versus normoxic rats (Figures 1B and 1C). MPs from other cellular origins were not significantly different between the two groups of rats, including those from leukocytes (CD45$^+$) and endothelial (CD54$^+$) cells (Figures 1D and 1E).

Hypoxic MPs Reduce NO Production in ECs

Control cells and those treated with MPs from either normoxic or hypoxic rats exhibited an electron paramagnetic resonance feature of signals derived from NO-Fe(DETC)$_2$. As shown in Figure 2A, treatment of aortic ECs with normoxic MPs did not affect NO production. However, hypoxic MPs significantly reduced NO release in ECs from aorta by approximately 45%. To identify the molecular mechanisms implicated in this decrease in NO production, we analyzed by Western blotting the expression levels and phosphorylation of eNOS as well as several enzymes linked to the NO pathway. In ECs from aorta, eNOS expression was not modified by MP treatment. By contrast, treatment by hypoxic MPs, but not by normoxic MPs, evoked a significant decrease in phosphorylation on the activator (Ser 1177) site (Figure 2B) and a significant increase in eNOS phosphorylation on the inhibitor (Thr 495) site (Figure 2C). Also, hypoxic, but not normoxic, MPs reduced phosphorylation of Akt on Ser 473 kinase involved in eNOS phosphorylation, without affecting its expression (Figure 2D). No effect of MPs was observed regarding caveolin-1 expression (Figure 2E).

In ECs from pulmonary arteries, hypoxic, but not normoxic, MPs reduced NO release (approximately 20% reduction) (Figure 3A). Although eNOS expression was not affected by MP treatment, hypoxic MPs induced a significant decrease in eNOS phosphorylation at Ser 1177 (Figure 3B) and a significant increase in eNOS phosphorylation at Thr 495 (Figure 3C).

![Figure 2](image-url)

**Figure 2.** Microparticles (MPs) from hypoxic rats decrease nitric oxide (NO) production in endothelial cells (ECs) from aorta. Cells were incubated for 24 hours in the presence of vehicle (Ctl), MPs from normoxic rats (NxMPs), or MPs from hypoxic rats (HxMPs). (A) Quantification of the amplitude of the NO-Fe(DETC)$_2$ signal in ECs from rat aorta. Values are expressed in units of amplitude/µg/mg of proteins of samples (n = 8–9). Histograms show the ratio of phosphorylation of (B) endothelial NO synthase (eNOS) Ser 1177 and (C) eNOS Thr 495 versus total eNOS, and (D) phospho-Akt Ser 473 versus total Akt and (E) caveolin-1. Immunoblots were quantified by densitometric analysis and normalized with either the full form of corresponding protein or with β-actin (for caveolin-1). Data are representative six to eight separate blots, and the densitometry values are given as mean ± SEM. *P < 0.05 and **P < 0.01.
However, in contrast to ECs from aorta, neither Akt phosphorylation on Ser 473 site nor its expression levels were changed (Figure 3D), whereas caveolin-1 expression was significantly reduced after hypoxic MP treatment (Figure 3E).

**Hypoxic MPs and \( \text{O}_2^- \) Release in ECs**

Electron paramagnetic resonance measurement of \( \text{O}_2^- \) production demonstrated that normoxic MPs did not modify \( \text{O}_2^- \) release from ECs from either the aorta or the extrapulmonary arteries (Figures 4A and 4B). By contrast, although hypoxic MPs did not affect \( \text{O}_2^- \) production in aortic ECs (Figure 4A), these MPs significantly increased \( \text{O}_2^- \) production by approximately 36% in ECs from pulmonary arteries compared with normoxic MPs (Figure 4B). To determine the source of ROS production, ECs from pulmonary arteries were incubated in the presence of inhibitors of xanthine oxidase (allopurinol), mitochondrial complex I (rotenone), or nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase (apocynin), and then, \( \text{O}_2^- \) production was evaluated. Allopurinol and rotenone significantly reduced the ability of hypoxic MPs to increase \( \text{O}_2^- \) production in ECs from pulmonary arteries. Interestingly, \( \text{O}_2^- \) production was not significantly different in the absence or in presence of apocynin after treatment of ECs from pulmonary arteries with hypoxic MPs (Figure 4C).

To strengthen the implication of xanthine oxidase, we quantified xanthine oxidase activity in ECs from pulmonary arteries treated with either normoxic or hypoxic MPs. As shown in Figure 4D, treatment of ECs from pulmonary arteries with hypoxic, but not normoxic, MPs induced an increase in xanthine oxidase activity when compared with control.

**Hypoxic MPs Impair Ex Vivo Endothelium-Dependent Relaxation**

To analyze whether the MPs can induce acute changes in pulmonary artery blood pressure, hypoxic MPs were injected into rats, and 24 hours later mean pulmonary artery blood pressure was recorded. Injection of hypoxic MPs did not significantly modify pulmonary artery blood pressure (values of pulmonary artery pressure being 15.6 ± 0.8 vs. 18.7 ± 2.7 mm Hg for normoxic noninjected rats (n = 15) and normoxic rats injected with hypoxic MPs (n = 4), respectively).

To study the ex vivo endothelial function, we assessed relaxation response to either acetylcholine in aorta or carbachol in extrapulmonary arteries from rats treated with hypoxic MPs. Injection of hypoxic MPs induced a rightward shift in the concentration–response curves to vasodilators in both aortic rings (pD2: 7.35 ± 0.04 vs. 6.72 ± 0.04 in normoxic vs. hypoxic MPs, \( P < 0.05 \)) (Figure 5A) and extrapulmonary arteries.

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*Figure 3.* Microparticles (MPs) from hypoxic rats decrease nitric oxide (NO) production in endothelial cells (ECs) from pulmonary arteries. Cells were incubated for 24 hours in the presence of vehicle (Ctl), MPs from normoxic rats (NxMPs), or MPs from hypoxic rats (HxMPs). (4A) Quantification of the amplitude of the NO-Fe(DETC)\(_2\) signal in ECs from rat pulmonary arteries. Values are expressed in units of amplitude/µg/µl of proteins of samples (n = 8–9). Histograms show the ratio of phosphorylation of (B) eNOS Ser 1177 or (C) eNOS Thr 495 versus total eNOS, and (D) phospho-Akt Ser 473 versus total Akt and (E) caveolin-1 expression. Immunoblots were quantified by densitometric analysis and normalized with either the full form of corresponding protein or with β-actin (for caveolin-1). Data are representative of six to eight separate blots, and the densitometry values are given as mean ± SEM. \( ^* P < 0.05 \) and \( ^{**} P < 0.01 \).
(pD2: 7.59 ± 0.04 vs. 6.66 ± 0.05 in normoxic vs. hypoxic MPs, P < 0.05) (Figure 5B), without affecting the maximal relaxation.

Finally, to determine whether the differential effects of MPs were due to qualitative difference or just due to increased levels of circulating MPs from hypoxic rats, we injected, in control rats, a twofold higher quantity of normoxic MPs to have the same number present as for hypoxic MPs. Only the injection of hypoxic MPs induced a rightward shift of the concentration–response curves to carbachol compared with injection with either vehicle or a double amount of normoxic MPs (pD2: 7.16 ± 0.04 for a double amount of normoxic MPs), without affecting the maximal relaxation (Figure 5B). These results suggest that the differential effect between normoxic and hypoxic MPs is mainly due to qualitative differences in MP composition.

DISCUSSION

In the present study, circulating levels of MPs were elevated in hypoxic rats compared with normoxic rats. It is particularly interesting to note that MPs were mainly derived from platelets and erythrocytes. We also demonstrate that hypoxic MPs decreased NO production in ECs from both aorta and pulmonary arteries, which was linked to a decrease in phosphorylation of eNOS at its stimulatory site and an increase in phosphorylation at its inhibitory site. On the other hand, O2− production was increased only in ECs from pulmonary arteries by a mechanism sensitive to allopurinol and rotenone and associated with an increase of xanthine oxidase activity. These results underscore a tissue specificity of the effects of MPs, depending on the vascular bed, in terms of oxidative stress. Furthermore, injection of hypoxic MPs decreased endothelium-dependent relaxation in systemic and pulmonary arteries. Altogether, these results suggest that hypoxic MPs induce endothelial dysfunction and highlight the involvement of MPs from hypoxic animals in the pathogenesis and in the maintenance of PAH by affecting mainly the NO pathway and oxidative stress in the pulmonary vasculature.

Although circulating levels of MPs are increased in patients with PAH (8, 9), differences in MP origin have been reported. Although only levels of MPs derived from ECs and leukocytes are increased in patients with PAH (9), Bakouboula and colleagues (8) have shown that procoagulant MPs are elevated in patients with PAH. Here we demonstrate that in an animal model of hypoxic PAH the number of MPs, mainly from platelets and red cells, is increased. These differential data can be explained by the difference in the heterogeneity of patients with PAH, by the different steps of centrifugation used for MP isolation, or by the different membrane markers used to identify cell origin of MPs. We cannot distinguish among these possibilities, although the model of hypoxic PAH used in the present study displays similar cardiovascular characteristic of PAH observed in patients, such as increased pulmonary vascu-
Hypoxic microparticles (MPs) impair endothelium-dependent relaxation. (A) Acetylcholine (Ach)-induced relaxation in aorta from control (Nx) and hypoxic (HxMPs) MP-injected rats. (B) Carbachol (CCh)-induced relaxation in pulmonary arteries from Nx, double amount of normoxic MP (2*NxMPs) and HxMPs-injected rats. Results were expressed as a percentage of relaxation of phenylephrine-induced precontraction. \(* * * P < 0.001.\)

Figure 5. Hypoxic microparticles (MPs) impair endothelium-dependent relaxation. (A) Acetylcholine (Ach)-induced relaxation in aorta from control (Nx) and hypoxic (HxMPs) MP-injected rats. (B) Carbachol (CCh)-induced relaxation in pulmonary arteries from Nx, double amount of normoxic MP (2*NxMPs) and HxMPs-injected rats. Results were expressed as a percentage of relaxation of phenylephrine-induced precontraction. \(* * * P < 0.001.\)

A prolonged exposure to hypoxia results in a decrease in aortic eNOS protein expression and an impaired endothelium-dependent relaxation to acetylcholine on aortic rings in rats (11). The role of eNOS in the PAH pathogenesis is well demonstrated; thus overproduction of eNOS in transgenic mice prevents hypoxia-induced PAH (21), whereas exposure to mild hypoxia results in severe PAH in eNOS-deficient mice (22). In the present study, in vitro endothelial function was assessed by direct measurement of NO production by ECs treated with MPs. We have shown a decrease in NO production in ECs from both aorta and pulmonary arteries treated with hypoxic MPs, indicating that hypoxic MPs might contribute to endothelial dysfunction observed in chronic hypoxia-induced PAH. In addition, with the changes in eNOS expression, NO production can be regulated by eNOS post-translational modifications.

When Ser 1177 is phosphorylated by several kinases, such as Akt (23), NO production is increased twice or three times compared with basal levels, whereas Thr 495 phosphorylation by protein kinase C is associated with a decrease in NO production (24). Thus the double phosphorylation of Ser 1177 and Thr 495 is crucial for eNOS activity in ECs. We found that hypoxic MPs were able to modify eNOS phosphorylation without affecting its expression both in ECs from aorta and pulmonary arteries. Indeed, hypoxic MPs increased Thr 495 and decreased Ser 1177 phosphorylations in ECs from both types of arteries. Furthermore, hypoxic MPs induced a decrease in Akt phosphorylation, Akt being the kinase involved in eNOS activation-associated phosphorylation in aortic ECs. Although eNOS phosphorylation by Akt has been reported to be impaired in hypoxic pulmonary arteries (25), hypoxic MPs were not able to modify Akt phosphorylation in ECs from pulmonary arteries under the experimental conditions used. Regarding the latter, other kinases such as AMP-activated protein kinase or protein kinase C (26, 27) may be implicated in the eNOS phosphorylation at the inhibitory site evoked by hypoxic MPs. Nevertheless, decreased eNOS activity probably concurs to reduce NO production in ECs from both aorta and pulmonary arteries.

The subcellular localization of caveolin-1 is critical for eNOS regulation. In ECs, caveolin-1 negatively regulates NO signaling by binding eNOS and sequestering it at the plasma membrane. The activation of caveolar eNOS depends on its dissociation from caveolin-1 (28); in addition, if the interaction between caveolin-1 and eNOS is altered, vascular integrity and function are affected. Here, we demonstrate a decrease in caveolin-1 expression only in ECs from pulmonary arteries treated with hypoxic MPs, which may argue against a reduced eNOS activity. However, in this context, several works have described an alteration in eNOS and caveolin-1 link in PAH leading to the alteration of vascular integrity and decrease in NO production. Thus, reduction in the expression of caveolin-1 has been reported in primary PAH and in experimental models of PAH induced by monocrotaline (29, 30) and hypoxic PAH (31). Altogether, pulmonary hypertension affects caveolin/eNOS balance, the consequence of which concurs with endothelial dysfunction. In line with these studies, we found that 24-hour treatment with hypoxic MPs induced both reduction in caveolin-1 expression and an increase in phosphorylation of eNOS on its inhibitor site leading to a decrease in NO production. Thus, the present data strengthen the notion that hypoxic MPs participate in the changes in endothelial integrity during PAH.

Superoxide anion can react with other reactive species, including NO. The $\text{O}_2^-$/NO interaction occurs at very high speed constant, which leads to NO inactivation and plays a major role in endothelial dysfunction associated with vascular pathologies (7). However, although the participation of ROS production in PAH pathogenesis is generally accepted (32), the sources responsible for ROS production in the hypoxic pulmonary arteries are not well defined (33). Several studies have suggested an involvement of the NADPH oxidase in the development of hyperreactivity and endothelial dysfunction during PAH (6, 34). In other models of PAH, an increased capacity for ROS production by NADPH oxidase as well as by mitochondria has been described (35, 36). Hoshikawa and colleagues (37) have found that in lung from adult rats, xanthine oxidase activity is elevated under hypoxic conditions, especially during the induction phase ($\sim 7$ d) of hypoxic exposure, because treatment with allopurinol in the first 3 days of hypoxia is sufficient to limit right ventricular hypertrophy and pulmonary vascular thickening. Most recently, in neonatal rats exposed to hypoxia, it has been reported that $\text{O}_2^-$ generation from...
xanthine oxidase is implicated in endothelial dysfunction and vascular remodeling (38). Our results demonstrate that O$_2^-$ production by treatment with hypoxic MPs takes place only in ECs from pulmonary arteries, indicating a tissue specificity of hypoxic MPs toward ECs from the pulmonary bed. In addition, these effects are associated with a participation of both xanthine oxidase and mitochondrial complex I, without implicating NADPH oxidase or changes in gp91 expression (data not shown). These data are in accordance with the implication of xanthine oxidase activity on the deleterious effects in endothelial function induced by MPs derived from apoptotic lymphocytes (15) and reveal the ability of hypoxic MPs to modulate oxidative status in pulmonary ECs.

Finally, although hypoxic MPs were not able to modify pulmonary blood pressure after 24 hours of in vivo treatment, they reduced the ability of muscarinic agonists to promote endothelium-dependent relaxation in both aorta and pulmonary arteries. These results highlight the distinction between endothelial dysfunction and changes in pulmonary blood pressure after treatment with hypoxic MPs for 24 hours. It should be noted that the increase in pulmonary blood pressure described in hypoxic animal models is associated with hypertrophy of smooth muscle cells resulting in an increased vascular resistance. Thus, one can advance the hypothesis that acute treatment with hypoxic MPs might not be able to affect smooth muscle proliferation.

In the present study, hypoxic MPs negatively affected the eNOS pathway, decreased NO release, and increased ROS production in ECs. These results are in concordance with other data present in the literature, wherein a decrease in endothelium-dependent relaxation is widely reported in pulmonary (6, 39) and systemic (11, 12) circulation during hypoxia.

In summary, we report that hypoxic rats developing PAH have increased levels of circulating MPs, especially platelet- and erythrocyte-derived MPs. Hypoxic MPs can impair endothelial function in both rat aorta and pulmonary arteries, by directly reducing eNOS activity and by limiting NO bioavailability, and, in the pulmonary bed only, by increasing ROS production. Together, these data strongly suggest that circulating hypoxic MPs are able to induce endothelial dysfunction and demonstrate for the first time their pathophysiologic relevance, in this model. From these results, one can advance the hypothesis that hypoxic MPs contribute to the pathophysiological process of PAH.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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