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REVIEW

Lung Mast Cells and Hypoxic Pulmonary Hypertension

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Summary

Hypoxic pulmonary hypertension (HPH) is a syndrome characterized by the increase of pulmonary vascular tone and the structural remodeling of peripheral pulmonary arteries. Mast cells have an important role in many inflammatory diseases and they are also involved in tissue remodeling. Tissue hypoxia is associated with mast cell activation and the release of proteolytic enzymes, angiogenic and growth factors which mediate tissue destruction and remodeling in a variety of physiological and pathological conditions. Here we focused on the role of mast cells in the pathogenesis of hypoxic pulmonary hypertension from the past to the present.

Key words

Pulmonary hypertension • Mast cells • Hypoxia • Tissue remodeling

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Introduction

Hypoxic pulmonary vasoconstriction (HPV) is a physiological mechanism optimizing the matching of ventilation and perfusion in the lung during acute hypoxia. Vasoconstriction causes an increase in pulmonary arterial pressure (Herget and Ježek 1989). The elevation of intracellular Ca^{2+} in pulmonary arterial smooth muscle cells plays the main role in HPV. ROS generation, voltage-gated K^+ channels (K_V), Ca^{2+} release from ryanodine-sensitive stores or Rho kinase-mediated Ca^{2+} sensitization are also involved, as discussed in detail in reviews (McMurtry *et al.* 2010, Ward and McMurtry 2009).

Chronic (weeks of) hypoxia leads to the development of hypoxic pulmonary hypertension (HPH). HPH is a syndrome characterized by the increase of pulmonary vascular tone and the structural remodeling of peripheral pulmonary arteries. These changes regress after the return to normoxia (Herget *et al.* 1978, Rabinovitch *et al.* 1981, Reid 1986).

The detailed mechanisms of HPV and then HPH development remain unexplained, but the Rho kinase pathway seems to be also responsible for the regulation of pulmonary vascular tone in chronic pulmonary hypertension (McMurtry *et al.* 2010). The second component of HPH – vascular remodeling – results from proliferation of pulmonary artery smooth muscle cells and/or their increased resistance to apoptosis (Dumas de la Roque *et al.* 2010).

Mast cells produce a vast amount of mediators with growth, proteolytic and proangiogenic effects and prove to be a very interesting population of bone marrow–derived cells involved in the pathogenesis of many diseases (Pejler *et al.* 2010). Recent articles (Dahal *et al.* 2011, Hoffmann *et al.* 2011, Montani *et al.* 2011) intensively studied the role of mast cells in the rat model of monocrotaline-induced pulmonary arterial hypertension and in idiopathic pulmonary arterial hypertension (IPAH).

This review summarizes research up to the present day about the role of mast cells in the pathogenesis of hypoxic pulmonary hypertension.

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Mast cells in acute hypoxia

Several investigators have searched for the causes of the different reactivity between systemic and pulmonary vessels during hypoxia. The first hypothesis was that HPV (initially termed as pulmonary pressor response to hypoxia) is mediated *via* the release or activation of a local hormone (Barer 1966, Robin *et al.* 1967). Many agents such as catecholamines, serotonin and histamine have been considered (Barer and McCurrie 1969, Hauge and Melmon 1968).

Serotonin and histamine

Pharmacological agents that block or potentiate the effect of vasoactive substances were studied in isolated and perfused lungs (Hauge 1968, Hauge and Melmon 1968), but neither the serotonin-blocking agent UML 491 nor lung perfusion with platelet rich plasma as the abundant source of serotonin affected HPV. Nor did a later experiment (Helgesen and Bjertnaes 1986) with ketanserin (5HT-2 specific blocker) in doses adequate to block the response to serotonin confirm the role of serotonin in HPV.

Aviado *et al.* (1966) described a significant rise in the histamine levels in venous blood from hypoxic lungs in dogs. Studies in isolated and perfused lungs (Hauge 1968, Hauge and Melmon 1968) showed that antihistamines of four different chemical classes abolished pressor response to alveolar hypoxia in isolated and ventilated lungs of rats, whereas a histaminase-inhibiting compound potentiated this response. The administration of the histamine releasing agent 48/80 caused a rise in pulmonary vascular resistance within seconds. They demonstrated that the level of tissue histamine is related to the magnitude of the hypoxic pulmonary vasoconstrictor response.

Source of histamine – mast cells

Researchers then focused on the source of histamine in pulmonary tissue – mast cells. Mast cells were discovered by Paul Ehrlich in 1878, who described them in his doctoral thesis at the University of Leipzig (Ehrlich 1878). Histamine in mast cells was identified much later in 1952 by James Riley and Geoffrey West (Riley and West 1952). For a historical overview see (Beaven 2009, Vyas and Krishnaswamy 2005).

The first study concerned with pulmonary mast cells and HPV came from Haas and Bergofsky (Haas and Bergofsky 1972). They found a predictable distribution of perivascular mast cells in small pulmonary arteries in rats, *in vivo* degranulation of pulmonary mast cells during alveolar hypoxia and a proportional rise in pulmonary vascular resistance with the released amount of histamine. Acute alveolar hypoxia did not alter the mast cell distribution and total number of visible mast cells.

But their results were not supported by the findings of others in isolated cat lung (Dawson *et al.* 1974), in rats (Kay *et al.* 1974) and dogs (Tucker *et al.* 1976). Kay *et al.* (1974) did not find any qualitative evidence of histamine depletion in the lungs of the rats exposed to acute hypoxia by using a histochemical assay for the demonstration of histamine in mast cells. Fluorescence microscopy revealed clear evidence of histamine depletion of perivascular mast cells only after the administration of compound 48/80. An experiment using a combination of H1- and H2-receptor blockers also did not prevent or reduce the hypoxic pulmonary pressor response in dogs (Tucker *et al.* 1976). It is important to say, that these findings were obtained using different animal models, techniques and experimental preparations. In rats, for example the degree of mast cells degranulation (grade 1-3) has been studied by light microscopy in work of Haas and Bergofsky (Haas and Bergofsky 1972), whereas Kay *et al.* (1974) used for measurement of histamine depletion histochemical examination by fluorescence microscopy. Kay *et al.* used more severe hypoxic stimulus for shorter time in comparison with previous work of Haas and Bergofsky.

Blockade of mast cells degranulation – effect on HPV

To investigate the role of mast cell products in HPV, researchers used a mast cell membrane stabilizing agent which prevents the release of mediators from the cells – cromolyn sodium. Cromolyn sodium is derived from chromone khellin extracted from herb *Ammi visnaga*. Its oral absorption is minimal and the effects in animal models may vary from species to species (Bernstein 1985). While Kay and Grover (1975) inhibited the pulmonary pressor response in three of eight anesthetized dogs; Howard *et al.* (1975) were not able to confirm this in cats and dogs. Frantz *et al.* (1988) in newborn and young lambs described that hypoxia-induced pulmonary hypertension was not inhibited by cromolyn sodium in either age group and that the mast cell products are not important mediators of hypoxia-induced pulmonary hypertension. But the term hypoxia-induced pulmonary hypertension is in this case misguiding, because all measurements were made after

10 min of hypoxic exposure. Thus they described effect of cromolyn sodium on HPV rather than on HPH.

Mast cells in chronic hypoxia

The lack of any strong evidence for a role of mast cells in acute hypoxic pulmonary vasoconstriction led to a change in focus to their potential role in chronic hypoxia.

Morphology and distribution in lungs

Some investigators found a higher total number of mast cells in the lungs of chronically hypoxic rats (Kay *et al.* 1974, Mungall 1976). Kay *et al.* described that the mast cells do not differ morphologically, but their distribution is altered (lower amount of peribronchial cells with an accumulation of the cells in the alveolar septa). There was a linear relation between the logarithm of the right ventricular weight and the logarithm of the lung mast cell density. Tucker *et al.* (1977) determined the influence of chronic hypoxia on mast cells in six species. They described significant changes in the lung density of mast cells in chronically hypoxic pigs, rats and sheep. Positive correlations of perivascular mast cell density and right ventricular hypertrophy they demonstrated in calves, pigs, rats and sheep.

Migally *et al.* (1983) showed increased proliferation of perivascular mast cells, as well as increased secretory activity of vasoactive substances in aging animals. They therefore suggested that mast cells may be responsible for the decrease in pulmonary vascular responsiveness to chronic hypoxia in aging rats when compared with young animals. Nadziejko *et al.* (1989) determined granule content of perivascular mast cells in hypoxic lungs. Alveolar hypoxia reduced the granular content by 12 % nevertheless hypoxic lungs did not show any morphological changes seen in IgE-mediated degranulation. Vajner *et al.* (2006) described the redistribution of the mast cells during chronic hypoxia and recovery from chronic hypoxia. Mast cells accumulate in the prealveolar portion of the pulmonary vasculature after 4 days of hypoxia. Later, when the HPH is fully developed, mast cells prevail in the conduit portion of the pulmonary vasculature. After 1 week of recovery in air, mast cells migrate and increase their numbers in the prealveolar and small muscular arteries.

The mechanism of increase in mast cell density in lungs could be similar as has been shown in cardiac pathologies: recruitment of hematopoetic precursor cells, maturation of immature resident cells and proliferation of resident cells (Levick *et al.* 2011).

Hypoxic pulmonary hypertension in mast cell-deficient animals

Because of conflicting data on the importance of mast cells in hypoxic pulmonary vasoconstriction and the development of hypoxic pulmonary hypertension, Zhu *et al.* (1983) examined these responses in a mast cell-deficient strain of mice W/W^V. This strain is infertile, has no lung mast cells and less than 1 % of the number of mast cells observed in the tissues of congenic +/+ mice (Kitamura *et al.* 1978). Zhu *et al.* did not find any differences between normal and deficient strains in the degree of acute or chronic pulmonary arterial hypertension, the increase in right ventricular weight and the remodeling of pulmonary arteries. They concluded that in mice, mast cells have no active role in HPV and HPH, but also observed that the normal mouse has many fewer mast cells compared with a rat and that the mouse is not an appropriate model of HPH.

Attenuation of pulmonary hypertension and vascular remodeling was described by Hoffmann *et al.* (2011) in Ws/Ws rats with congestive heart failure. This mutant strain has deletion in the tyrosine kinase domain of the c-kit gene (Tsujimura *et al.* 1991), the rats exhibit hypoplastic anemia and are fertile. Ws/Ws rats are usually used for the study of the effect of mast cells in myocardial remodeling (Boerma *et al.* 2008, Kennedy *et al.* 2005, Levick *et al.* 2008). Hoffmann *et al.* (2011) compared Ws/Ws rats to wild types. The increase in PAP and PVR following aortic banding was significantly attenuated in Ws/Ws rats, right ventricular hypertrophy was reduced, and the increase in medial wall thickness and muscularization and the loss of vessel lumen was markedly attenuated in banded Ws/Ws rats.

Blockade of mast cells degranulation – effect on HPH

Mungall (1976) investigated the effect of cromolyn sodium on the development of right ventricular hypertrophy during chronic hypoxia in rats. He postulated that the blockade of the mast cell degranulation prevents the development of pulmonary hypertension and thus prevents right ventricular hypertrophy. He did not measure pulmonary arterial pressure, but he found that disodium cromoglycate had no protective effect against right ventricle hypertrophy. Kay *et al.* (1981) described less right hypertrophy in cromolyn treated chronically hypoxic rats. Banasova *et al.* (2008) confirmed this effect

of the administration of disodium cromoglycate (DSCG) during the early phase of chronic hypoxia and then the role of mast cells in the development of HPH in rats. The blockade of mast cell degranulation also affects the restoration of pulmonary arterial pressure in the recovery from chronic hypoxia in rats (Maxova *et al.* 2010c). These contradictory results are probably due to use of different models of HPH and dose of disodium cromoglycate. While Mungall (1976) exposed rats to long lasting intermittent isobaric hypoxia and described changes in RV after as much as 70 exposures, others used continual hypobaric or isobaric hypoxia and they demonstrated RV hypertrophy in non-treated rats after 20 days. The dose of disodium cromoglycate used by Mungall was four-times lower than in experiment of Banasova *et al.* (2008).

Hoffmann *et al.* (2011) used other mast cell stabilizer – ketotifen in the rats with left heart disease and in the rats with monocrotaline-induced pulmonary arterial hypertension. Ketotifen attenuated pulmonary hypertension and vascular remodeling in both cases.

Mast cells and tissue remodeling

Mediators involved in remodeling

Mast cells are known as the main effector cells in the IgE-mediated allergic response (bronchial asthma, urticaria, allergic rhinitis) *via* the FcεRI receptor (Metcalfe *et al.* 1997). Activation of tyrosine kinases is central to the ability of both FcεRI and growth factor receptor KIT to transmit downstream signaling events required for the regulation of mast cell activation (Gilfillan and Rivera 2009). Although this is the classical pathway, other mechanisms of mast cell activation exist, such as complement, ligands of toll-like receptors, cytokines, endogenous peptides and physical stimuli (Galli and Tsai 2008) which can play a role in a number of non-allergic immune reactions (Maltby *et al.* 2009) such as tumor growth and angiogenesis (Ribatti *et al.* 2009), wound healing (Fajardo and Pejler 2003) or tissue remodeling (Galli and Tsai 2008).

Mast cells, upon appropriate activation, produce a variety of mediators classified as "preformed" mediators: histamine, serotonin, matrix metalloproteinases, proteases – tryptase, chymase and carboxypeptidase A, proteoglycans, preformed TNF-α and "de novo synthetized" derivates of arachidonic acid – prostaglandins and leukotrienes or later produced cytokines, chemokines and growth factors (Galli and Tsai 2008, Gordon and Galli 1991, Norrby 2002).

Some of these mediators are involved in tissue remodeling, such as serine proteases – tryptase, chymase and matrix metalloproteinases (MMPs). These proteolytic enzymes along with angiogenic and growth factors mediate tissue destruction and remodeling in a variety of physiological and pathological conditions, including airway remodeling in asthma (Karakiulakis *et al.* 2007), myocardial hypertrophy (Levick *et al.* 2008), chronic liver diseases (Franceschini *et al.* 2006), chronic pancreatitis (Esposito *et al.* 2001), remodeling of pulmonary vessels in chronic hypoxia (Banasova *et al.* 2008, Novotna and Herget 2002), or in recovery from chronic hypoxia (Maxova *et al.* 2010c, Tozzi *et al.* 1998).

MMPs are regulated in the lung *via* many factors as growth factors, hormones, cytokines, cell adhesion molecules or ECM proteins (Greenlee *et al.* 2007). For example, mechanical stress produces ROS in vascular smooth muscle cells and increase gene expression of MMP-2 (Grote *et al.* 2003), gelatinases MMP-2 and MMP-9 are activated by chymase, mice lacking the mast cell chymase fail to process proMMP-2 and proMMP-9 to their active forms (Tchougounova *et al.* 2005). Inhibitory regulation of MMPs activity is provided by a non-specific inhibitor as α2-macroglobulin (Maskos 2005) or by specific inhibitors, the family of tissue inhibitors of metalloproteinases (TIMPs) (Novotna and Herget 2002). TIMPs modulate MMPs activities, and the balance between them is thought to determine the turnover of matrix proteins.

Hypoxia generates oxidant stress (Nakanishi *et al.* 1995), which plays an important role in the pathogenesis of HPH (Hampl and Herget 2000). Lachmanova *et al.* (2005) showed that administration of antioxidants in early phase of exposure to hypoxia attenuated the structural remodeling of peripheral pulmonary vessels and development of HPH. As was described both systemic hypoxia (Steiner *et al.* 2003) and ischemia-reperfusion injury (Singh and Saini 2003) result in mast cell degranulation. Both hypoxia and ischemia reperfusion are accompanied by increased formation of reactive species which can modulate mast cell activation. For the role of ROS and RNS in mast cells activation see review (Swindle and Metcalfe 2007).

KIT and FcεRI – mediated mast cell activation is regulated by tyrosine kinase network (Gilfillan and Rivera 2009). The Src-family protein kinase Fyn activation after hypoxia-dependent free radical production plays a key role on the secretion of angiogenic

factors as VEGF (Garcia-Roman *et al.* 2010). Hydrogen peroxide promotes IL-4 and IL-6 mRNA production and potentates FcεRI -induced cytokine release in RBL-2H3 cells (Frossi *et al.* 2007). Gullikson *et al.* (2010) studied human mast cell survival, degranulation and cytokine release in hypoxia (1 % O_2). Their data suggested that hypoxia does not induce spontaneous mast cell degranulation, provokes IL-6 secretion in mast cells and that mast cells are viable for several days of hypoxia.

Little data is available on the direct influence of hypoxia on synthesis and release of mast cells proteolytic enzymes involved in tissue remodeling. Hypoxia (10 % O_2) stimulates the formation of rodent-like interstitial collagenase MMP-13 in RBL-2H3 mast cells and the production of matrix metalloproteinases (MMPs) and tryptase in isolated rat lung mast cells (Maxova *et al.* 2008). Antioxidant N-acetylcysteine significantly reduces this reaction (Maxová *et al.* 2004). Hypoxia (3 % O_2) increases the production of both specific mast cells and the serine proteases tryptase and chymase in RBL-2H3 mast cells (Maxova *et al.* 2010b). MMPs formation is a highly regulated process; mast cell chymase can act as an activator of MMPs (Greenlee *et al.* 2007). Mast cells exposed to hypoxia are more capable of splitting collagen I and then facilitate the growth of vascular smooth muscle cells (Maxova *et al.* 2010a).

Pulmonary vessel wall remodeling

The involvement of matrix-degrading enzymes in pulmonary vessel wall remodeling was described first by Thakker-Varia *et al.* (1998). They observed a short-term increase in total proteolytic, collagenolytic and gelatinolytic activities in pulmonary arteries after return to normoxia. In the same year this group showed that immunoreactive collagenase is increased in the vessel wall of hypertensive rats and that this collagenase is predominantly localized in the connective tissue-type mast cells (Tozzi *et al.* 1998). Riley *et al.* (2000) described the accumulation of mast cells containing collagenase in remodelled pulmonary arteries. They proposed that the role of MMPs as a major extracellular pathway of collagenolysis and serine proteases occurred in elastolysis in lung vasculature. Vajner *et al.* (2006) confirmed that hypoxia increases the amount of rodent-like interstitial collagenase (MMP-13) in mast cells accumulated in the small prealveolar pulmonary arteries.

Novotna and Herget (1998) observed qualitative changes of collagen in the peripheral pulmonary arteries of hypoxic rats. Collagen fragments were identified by western blot analysis as 1/4 and 3/4 fragments of collagen type I (Herget *et al.* 2003) which are typical cleavage products of triple helicase activity of rat interstitial collagenase MMP-13 (Knauper *et al.* 1996). Fig. 1 shows the schema illustrating the pathogenesis of HPH.

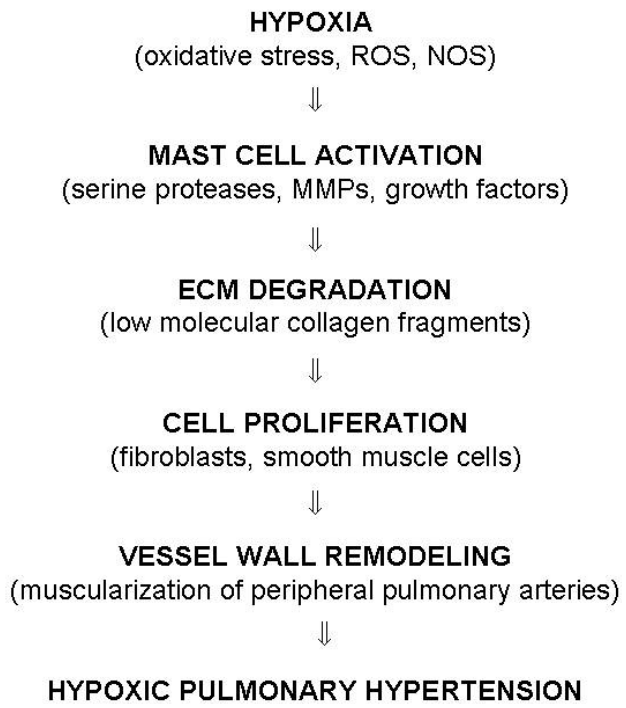

Fig. 1. Scheme illustrating the role of hypoxia activated mast in pathogenesis of HPH.

Pharmacological attenuation of HPH

As was described previously, the cellular sources of MMPs may include vascular smooth muscle cells (Jacob *et al.* 2001) and mast cells (Tozzi *et al.* 1998). Batimastat is a broad-spectrum MMPs inhibitor that reduces tissue injury (Chapman *et al.* 2003, Muhs *et al.* 2003). Collagen production is stimulated by activated mast cells in an MMPs dependent manner (Margulis *et al.* 2009). Batimastat inhibits intimal thickening after arterial injury by decreasing SMC migration (Zempo *et al.* 1996).

On the other hand Vieillard-Baron *et al.* (2000) reported that MMPs inhibition by doxycycline treatment was in rat model of hypoxic pulmonary hypertension associated with a significant increase in distal pulmonary artery muscularization. In contrast, Shi *et al.* (2009) using the same dose of doxycycline concluded that it attenuated pulmonary vascular remodeling and pulmonary hypertension in four different rat models of pulmonary hypertension. Vieillard-Baron *et al.* also investigated the effect of other MMP inhibition (intratracheal instillation

of the adenovirus-mediated human TIMP-1 gene). This treatment attenuated pulmonary hypertension in monocrotaline-induced model only (Vieillard-Baron *et al.* 2003) while it resulted in more pronounced muscularization of distal pulmonary arteries in hypoxia induced hypertension (Vieillard-Baron *et al.* 2000). MMPs, therefore, may have opposite effect in different models of pulmonary hypertension.

Herget *et al.* (2003) described the reducing effect of Batimastat on HPH in chronically hypoxic rats. The administration of Batimastat on each day of hypoxia reduced pulmonary arterial pressure, the percentage of double laminated peripheral pulmonary vessels and right ventricular hypertrophy. Treatment with this inhibitor prevented an increase in collagenolytic activity (MMP-2, MMP-9 and MMP-13) in peripheral pulmonary arteries.

Increased serine elastase activity has been implicated in both the monocrotaline-induced model of PAH (Todorovich-Hunter *et al.* 1992, Ye and Rabinovitch 1991) and in vascular remodeling associated with chronic hypoxia-related pulmonary hypertension (Zaidi *et al.* 2002). Elastase inhibitors suppress tenascin-C and induce smooth muscle cell apoptosis. Peptidyl trifluoromethylketone serine elastase inhibitors M249314 or ZD0892 initiated a complete regression of the hypertrophied vessel wall by a coordinated loss of cellularity and extracellular matrix (Cowan *et al.* 2000).

The role of lung mast cell collagenolytic activity in hypoxic pulmonary hypertension (Banasova *et al.* 2008) and in recovery from chronic hypoxia (Maxova *et al.* 2010c) was tested by the inhibitor of mast cell degranulation – disodium cromoglycate (DSCG). Administration of DSCG at the early phase of chronic hypoxia reduced the development of HPH, while treatment at the end of hypoxic exposure had no effect on the decrease of pulmonary arterial pressure and the percentage of double laminated peripheral pulmonary vessels. Early DSCG administration also inhibited the presence of collagen cleavages in the walls of peripheral pulmonary arteries. Preventive administration of mast cell stabilizer (Cromolyn) significantly attenuated right ventricular systolic pressure and improved muscularization of distal pulmonary vessels also in rat monocrotaline PH (Dahal *et al.* 2011). Similarly treatment with Cromolyn by a therapeutic approach had no effect on hemodynamics and vascular remodeling.

DSCG administration in chronically hypoxic rats with fully developed HPH, prevented the decrease of pulmonary arterial pressure and collagen splitting at the early phase of recovery. We propose that the effect of DSCG (Fig. 2) is mainly due to the prevention of the release of serine proteases, MMPs and growth factors from activated mast cells which can play a role in vascular remodeling.

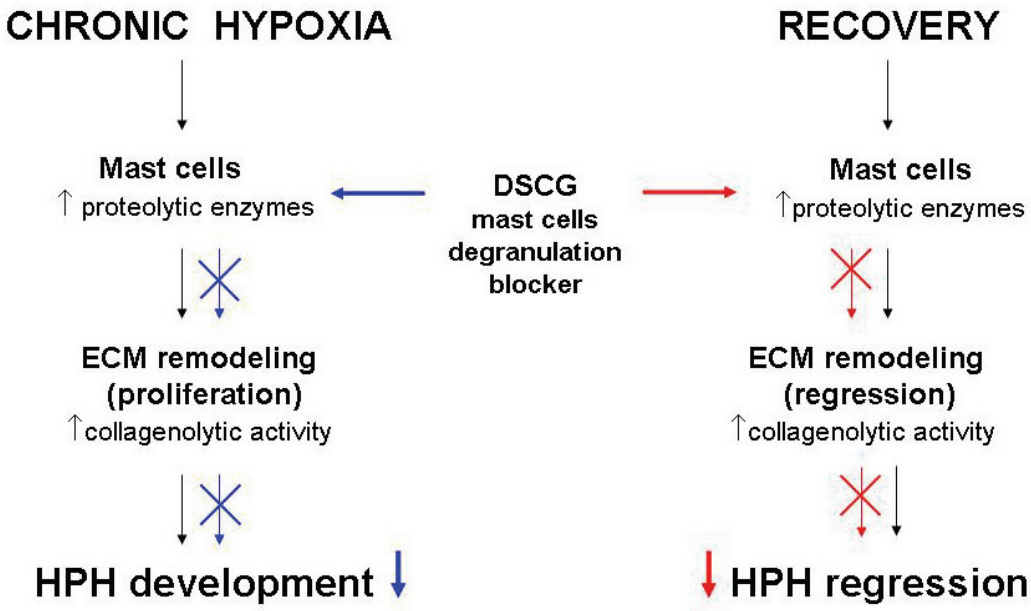

Fig. 2. Scheme shows the effect of mast cell degranulation blocker disodium cromoglycate (DSCG) on the development of hypoxic pulmonary hypertension and on the regression phase in recovery from chronic hypoxia. DSCG prevents release of proteolytic enzymes which plays a role in extracellular matrix (ECM) remodeling in both phases as was described in our works (Banasova *et al.* 2008, Maxova *et al.* 2010c).

Mast cells in other types of pulmonary hypertension

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by proliferation of endothelial and smooth muscle cells, fibrosis and inflammation. Monocrotaline in the rat induces pulmonary hypertension characterized by muscularization of the small pulmonary arteries (Okada *et al.* 1997). Although the pathogenesis of PAH differs from HPH, the remodeling of pulmonary vessels occurs in both circumstances. Van Albada *et al.* (2010) described increase in number of mast cells in intraacinar and preacinar vessels and higher expression of tryptase, chymase, mast cell protease 8 and IgE-Fc receptor in monocrotaline induced PAH. Accumulation of mast cells in lungs was described also in patients with idiopathic pulmonary arterial hypertension (IPAH) (Heath and Yacoub 1991). Contribution of mast cells to vascular remodeling and pulmonary hypertension of different etiologies confirmed Hoffmann *et al.* (2011). The recent study of Dahal *et al.* (2011) confirms accumulation of perivascular mast cells with enhanced degranulation in both IPAH patients and monocrotaline rat model of PH as compared to the healthy controls. Infiltration of c-kit positive cells in pulmonary arterial lesions with an increase in c-kit mRNA expression in human IPAH showed Montani *et al.* (2011).

Conclusions

Chronic hypoxia increases the production of proteolytic enzymes in mast cells which are more able to split collagen and facilitate the growth of vascular smooth muscle cells. Administration of a serine proteases inhibitor or mast cell degranulation blockers at the onset of exposure to chronic hypoxia attenuates the development of hypoxic pulmonary hypertension in experimental animals. Mast cell degranulation, release and activation of mediators participating in processes of tissue remodeling appear to be an important factor in the initial phase of hypoxia.

Conflict of Interest

There is no conflict of interest.

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