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Prevention of Mast Cell Degranulation by Disodium Cromoglycate Attenuates the Development of Hypoxic Pulmonary Hypertension in Rats Exposed to Chronic Hypoxia

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Key Words
Hypoxic pulmonary hypertension · Disodium cromoglycate · Mast cell · Pulmonary vascular remodeling · Matrix metalloproteinases

Abstract
Background: Chronic hypoxia induces lung vascular remodeling, which results in pulmonary hypertension. Vascular remodeling is associated with collagenolysis and activation of matrix metalloproteinases (MMPs). One of the possible sources of MMPs in hypoxic lung are mast cells. ***Objective:*** The role of lung mast cell collagenolytic activity in hypoxic pulmonary hypertension was tested by the inhibitor of mast cell degranulation disodium cromoglycate (DSCG). ***Methods:*** Rats were treated with DSCG in an early or later phase of isobaric hypoxia. Control groups were exposed to hypoxia only or to normoxia. Lung hemodynamics, muscularization and collagen metabolism in the walls of peripheral pulmonary vessels in the lungs were measured. ***Results:*** DSCG applied at an early phase of exposure to hypoxia reduced the development of pulmonary hypertension, inhibited muscularization in peripheral pulmonary arteries and decreased the amount of collagen cleavage fragments in prealveolar vessels. ***Conclusions:*** Mast cell degranulation plays a role in the initiation of hypoxic pulmonary vascular remodeling.

Introduction

Chronic hypoxia results in hypoxic pulmonary hypertension (HPH) characterized by fibrotization and muscularization of the walls of peripheral pulmonary arteries. The beginning of vascular remodeling is associated with increased collagen turnover due to elevated formation and activation of specific matrix metalloproteinases (MMPs) [1]. Their activity results in the deposition of low molecular weight fragments of collagen in the vessel wall [2]. These fragments (matrikines) then stimulate the proliferation of vascular smooth muscle cells and fibroblasts [3]. The increased collagenolytic activity is one of the causative factors in the pathogenesis of HPH since MMP inhibition by administration of their specific inhibitor,

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batimastat, markedly attenuated the development of HPH and partly prevented the thickening of walls of peripheral pulmonary arteries [4].

One of the possible sources of MMPs in hypoxic lungs are mast cells. Lung mast cells (LMCs) concentrate at the onset of hypoxic exposure close to the walls of prealveolar vessels and increase the formation of proteases including collagen-cleaving MMP-13 [5]. Moreover, MMP-13 (rodent-type interstitial collagenase), which plays a crucial role in cleaving native collagen, is produced by mast cells isolated from rat lungs exposed to hypoxia in vitro [6].

All these data support the hypothesis that LMC-derived MMPs play a role in the pathogenesis of HPH. Therefore, this study was designed to test whether administration of disodium cromoglycate (DSCG) – a drug that blocks LMC degranulation and may therefore inhibit the MMP release – will affect the development of HPH.

Material and Methods

Study Design

Four groups of adult male Wistar rats (Anlab, Prague, Czech Republic) were used. Experiments were performed in accordance with the European Community and NIH guidelines for using experimental animals. All procedures were approved by the Animal Studies Committee of our institution.

Three groups of rats were placed in an isobaric hypoxic chamber (F_iO_2 0.1) [7] for a period of 4 days or 3 weeks. In 2 groups exposed to hypoxia, DSCG (40 mg/kg b.w. i.p. once a day; cromolyn sodium salt, Sigma Aldrich, Prague, Czech Republic) was administered either at an early phase of exposure to chronic hypoxia (first 4 days, group DSCG + H, n = 13) or at a later phase (last 4 days, group H + DSCG, n = 8). The third hypoxic group was untreated (group H, n = 13). The normoxic group that served as control (group N, n = 13) was kept in air. Two studies were performed.

Hemodynamic Measurements

After 3 weeks of hypoxic or normoxic exposure in animals of the N (n = 8), H (n = 8), H + DSCG (n = 8) and DSCG + H (n = 8) groups, pulmonary arterial blood pressure (PAP, mm Hg) was recorded in rats anesthetized with thiopental (40 mg/kg b.w. i.p.; ICN Czech Republic, Roztoky, Czech Republic) and spontaneously breathing room air, using a catheter inserted in the pulmonary artery via the right jugular vein [4, 8]. Systemic arterial blood pressure (mm Hg) was measured in the cannulated left carotid artery. Cardiac output (ml/min) was estimated by ultrasonic flow probe placed at the ascending aorta after opening the chest under mechanical ventilation with room air [9]. After the measurements of hemodynamics, the heart and lungs were removed from the chest. The right heart ventricle (RV) and the left ventricle plus septum (LV + S) were separated and weighted. The lungs were filled with formol solution through the trachea and stored in formol for 4 weeks. Lung sections were then stained by the hematoxylin resorcin-fuchsin method, and the percentage of double-laminated peripheral vessels was counted as described previously [4, 10].

Collagen Composition of the Walls of Peripheral Pulmonary Arteries

An additional 5 animals from the N, H and DSCG + H groups were anesthetized with thiopental (40 mg/kg b.w. i.p.) after 4 days of hypoxia and euthanized by exsanguination from the abdominal aorta. Their lungs were removed from the chest, the 3rd and 4th branches of the pulmonary artery were dissected, digested by pepsin, and the supernatant with collagenous proteins was analyzed by SDS-PAGE electrophoresis. The 4-day period of hypoxia was selected because we knew from our previous studies that collagen cleavage is most abundant at the early phase of the hypoxic exposure [2].

Statistics

Statistical analyses were performed using ANOVA with Fischer's post-hoc test. Values of $p < 0.05$ were considered significant. The results are presented as means ± SE.

Results

Rats exposed to hypoxia had significantly lower body weight than controls (table 1), although before the exposure, their body weight did not differ.

PAP was elevated in all groups exposed to 3 weeks of hypoxia; however, in the group of rats treated with DSCG during the first 4 days of hypoxia, the increase was significantly lower ($p < 0.05$) than in the other 2 groups exposed to hypoxia (fig. 1). Neither hypoxia nor administration of DSCG affected systemic arterial blood pressure. Cardiac output was significantly lower in all hypoxia-exposed groups than in controls ($p < 0.001$). No differences in cardiac output were seen between the hypoxic groups (table 1).

Early application of DSCG partly prevented the thickening of the walls of peripheral pulmonary arteries in chronic hypoxia. The percentage of double-laminated peripheral pulmonary vessels was significantly lower ($p < 0.0001$) in the DCSG + H group than in the H and H + DSCG groups (fig. 2). The RV/LV + S ratio was significantly lower in both DCSG-treated groups compared with the nontreated group H ($p < 0.05$) (table 1). The values of the heart weight (RV + LV + S) did not differ between the groups (N = 999 ± 20, H = 1,056 ± 36, DCSG + H = 1,035 ± 44 and H + DSCG = 976 ± 36 mg). The weights of the RV were significantly higher ($p < 0.001$) in all groups exposed to hypoxia than in normoxic controls (N = 196 ± 5, H = 372 ± 24, DCSG + H = 329 ± 25 and H + DSCG = 309 ± 18 mg). The weights of the

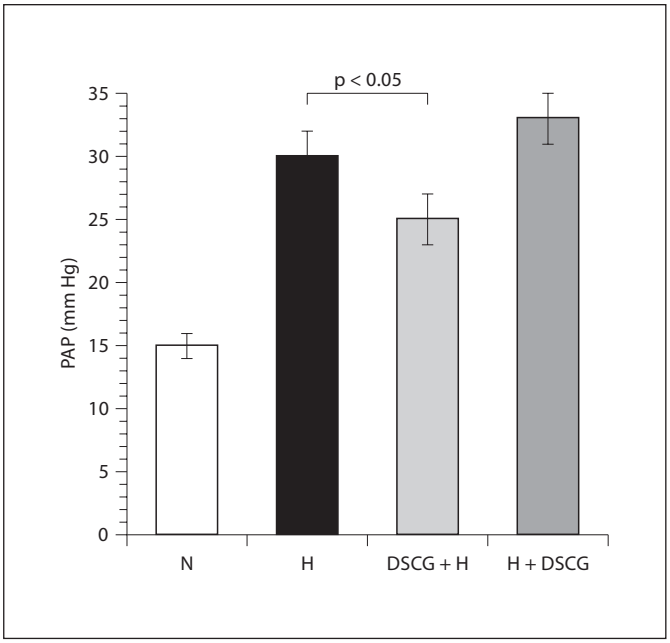

Fig. 1. DSCG treatment during the first 4 days of chronic hypoxia reduces PAP at the end of 3-week exposure. All groups exposed to hypoxia were significantly different ($p < 0.0001$) from the N group. $p < 0.05$ between the H group and the DSCG + H group.

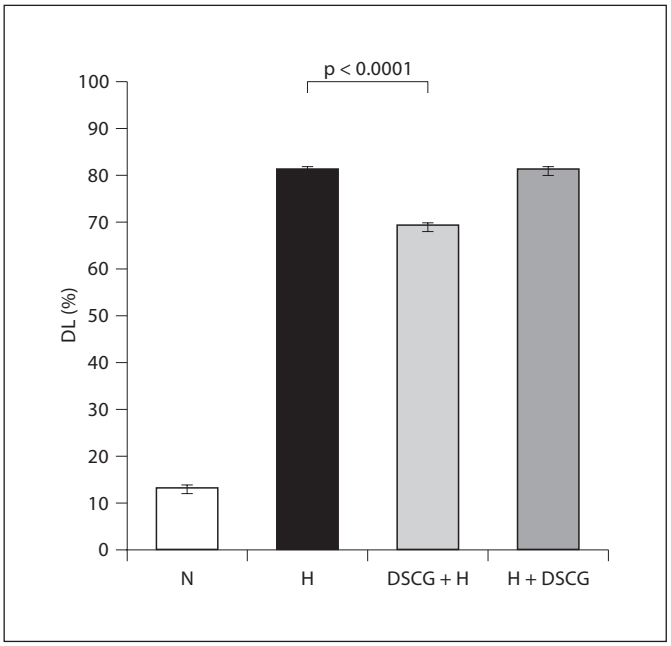

Fig. 2. DSCG treatment during the first 4 days of chronic hypoxia decreases the percentage of double-laminated peripheral pulmonary vessels (DL). All groups exposed to hypoxia were significantly different ($p < 0.0001$) from the N group. $p < 0.0001$ between the DSCG + H and the H group.

Table 1. Hemodynamic parameters

Group	n	Body weight, g	CO, ml/min	SAP, mm Hg	RV/LV + S, mg/g
N	8	425 ± 8	61 ± 3	114 ± 4[a]	0.245 ± 0.006
H	8	337 ± 5**	37 ± 3**, [a]	112 ± 4	0.541 ± 0.033**
DSCG + H (early)	8	329 ± 2**	38 ± 3**, [b]	111 ± 5	0.467 ± 0.028*, **
H + DSCG (late)	8	349 ± 7**	35 ± 2**	118 ± 4	0.470 ± 0.020*, **

The data are means ± SE. CO = Cardiac output; SAP = systemic arterial mean blood pressure.
* $p < 0.05$, early and late DSCG-treated groups versus the H group.
** $p < 0.001$, hypoxic groups versus the N group.
[a] Data from 7 animals; [b] data from 6 animals.

LV + S did not differ in groups exposed to hypoxia and they were lower ($p < 0.01$) than in normoxic rats (N = 802 ± 17, H = 685 ± 16, DCSG + H = 705 ± 25 and H + DSCG = 667 ± 31 mg).

Analysis of collagenous extracts from the walls of pulmonary arteries isolated from DSCG-treated and non-treated rats exposed for 4 days to hypoxia showed presence of the characteristic 3/4 and 1/4 fragments α1 and α2 chains of collagen type I in all groups. These fragments were present in the normoxic group in minute amounts, while the hypoxic groups showed substantial quantities (fig. 3a). Densitometric analysis of the fragments confirmed a significantly lower density of fragments in extracts from the group treated with DSCG compared with other animals exposed to hypoxia (fig. 3b).

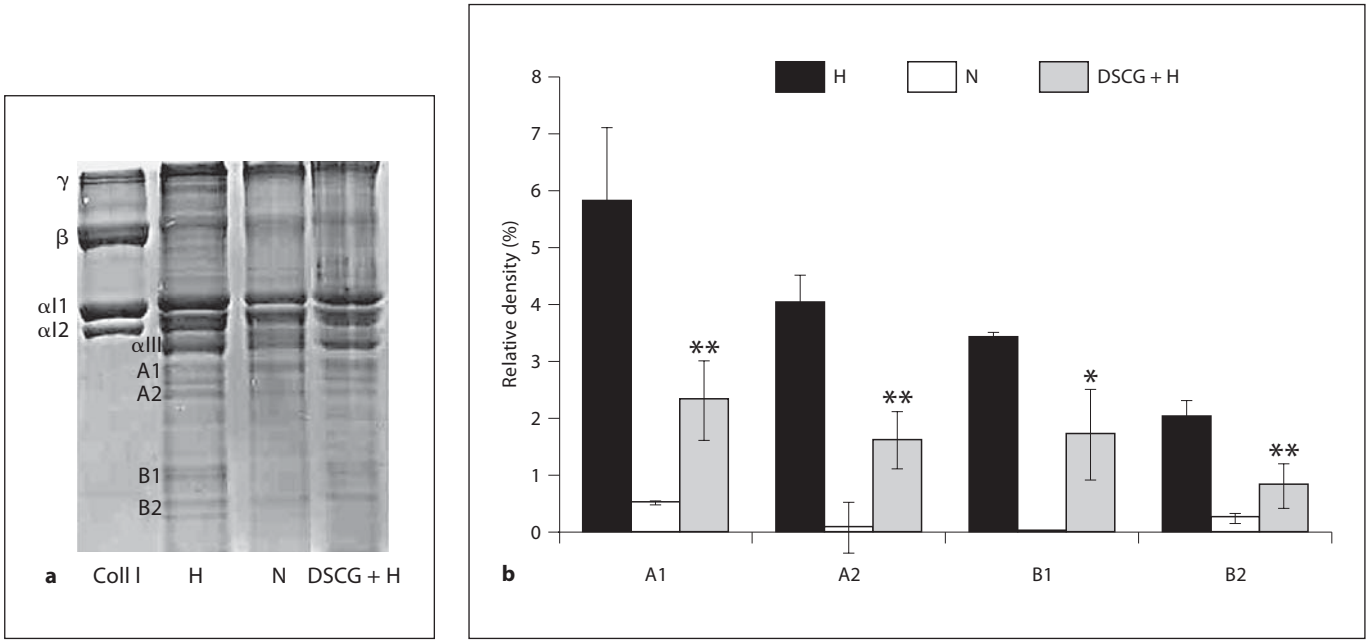

Fig. 3. a Gel electrophoresis profile of collagenous extracts from peripheral pulmonary arteries shows less collagen fragments in the DSCG + H group. Coll I = Standard of collagen type I from rat tail. **b** Relative densities of collagen fragments in extracts from peripheral pulmonary arteries. A1 and B1 (3/4 and 1/4 fragments) of α1 chain, A2 and B2 (3/4 and 1/4 fragments) of α2 chain in the groups H, N and DCSG + H. * $p < 0.02$ and ** $p < 0.01$, DSCG + H group versus H group.

Discussion

The main finding of our study is that prevention of mast cell degranulation by DSCG during the first 4 days of exposure to chronic hypoxia significantly attenuates the development of HPH and inhibits the presence of collagen cleavages in the walls of peripheral pulmonary arteries. Although mast cells produce various mediators, we suppose that the effect of DSCG is at least partly due to prevention of MMP release because the pharmacological inhibition of hypoxia-induced collagenolysis attenuates the development of HPH [4].

The fact that only early administration of DSCG had an inhibitory effect on pulmonary arterial hypertension gives support to the theory that the first days of hypoxic exposure play a crucial role in the pathogenesis of chronic HPH. In the rat, the HPH develops during the first 1–2 weeks of hypoxic exposure. Then HPH levels off into a steady state and does not progress any further. The first week of hypoxic exposure is characterized by radical damage of the lung tissue [11], and the lung mast cells accumulate in the vicinity of prealveolar pulmonary arteries particularly in the first week of exposure. In the steady state, after 3 weeks of exposure, most mast cells were found near the conduit portion of the pulmonary vasculature [5]. We hypothesize that remodeling of prealveolar pulmonary arteries starts with an increased activity of the interstitial collagenase MMP-13 (rodent-like interstitial collagenase), which cleaves native collagen into 3/4 and 1/4 fragments. These fragments are typically present in peripheral pulmonary arteries of hypoxic animals [12] and, as reported by Gardi et al. [13], they can stimulate lung collagen metabolism. We show that the amount of these collagen fragments is reduced by inhibition of mast cell degranulation.

Mast cells degranulate in alveolar hypoxia [14]. Other authors [15] inhibited mast cell degranulation in rats with DSCG given during the whole period of exposure to hypoxia and found less right heart hypertrophy than in nontreated hypoxic rats, similarly to our study with DSCG treatment for only part of the exposure. The positive results in that study were the reason why we selected DSCG from the battery of drugs known to inhibit mast cell degranulation. In contrast, Mungall [16] did not demonstrate a protective effect of DSCG on the right ventricular hypertrophy in rats exposed to hypoxia. However,

the used dose of DSCG was 4 times lower than in our experiment (10 mg/kg b.w. i.p.). Zhu et al. [17] did not observe differences in PAP, right ventricular hypertrophy and peripheral pulmonary vessel muscularization between intact and mast cell-deficient mice exposed to chronic hypoxia. In contrast to the observations from our group [5], they did not find any effect of chronic hypoxia on the number or appearance of mast cells in control (mast cell possessing) mice. As a possible explanation, they argue by the relative infrequency of mast cells in the normal mouse compared with the rat. Tucker et al. [18] observed a wide variability in the number of lung mast cells between species. The number of mast cells correlated positively with the severity of HPH.

DSCG is a compound that inhibits the release of mediators from mast cells and acts as an anti-inflammatory agent [19]. The mechanism of action is not fully understood, but it is probably mediated by the phosphorylation of a 78-kDa mast cell protein, which leads to positional rearrangements of the membrane cytoskeleton and approaches plasma and secretory granule membranes [20]. By this mechanism, DSCG could inhibit the release of all mediators including chymotrypsin-like proteases, growth factors and chemotactic factors. Steiner et al. [21] demonstrated that blockade of mast cell degranulation significantly inhibited the microvascular response to systemic hypoxia (e.g., hypoxia-induced increase in reactive oxygen species, adherence and migration of leukocytes and vascular permeability). All these mechanisms participate in the pathogenesis of HPH [22].

Repeated lung inflammations with episodes of lung hypoxia are an important cause of HPH in patients [23–25]. Inhibitors of mast cell degranulation may mitigate the development of HPH in these patients.

As expected, the RV/LV + S weight ratio increased in our H group (rats exposed to chronic hypoxia only), and this increase was attenuated in our group treated with DSCG at the early stage of the hypoxic exposure along with the inhibition of the increase in PAP. Surprisingly, the ratio was similar in both DSCG-treated groups, i.e. administration of DSCG for the last 4 days of hypoxia affected the RV/LV + S weight ratio although it had no effect on PAP. This finding could be explained by different effects of hypoxia on mast cell density in the heart and peripheral pulmonary vessels. An increase in the mast cell density in the vicinity of prealveolar pulmonary blood vessels is restricted only to the early phase of hypoxic exposure. After 3 weeks of hypoxia, the number of mast cells in that location does not differ from that found in normoxia [5]. This explains why DSCG applied at the end of hypoxic exposure has no effect on pulmonary vascular resistance and vascular remodeling. In contrast, Rakusan et al. [26] reported a persistent increase in the number of mast cells in hearts of rats acclimatized to high altitude for 4 weeks. The interesting finding of their study is that a similar increase in the heart mast cell density was not found in rats with renal hypertension. It suggests that the increase in heart mast cell density is not a simple function of ventricular pressure load.

We conclude that administration of DSCG at an early stage of hypoxia reduced the development of HPH while it is without effect if administered at a later stage of hypoxic exposure. Mast cell degranulation appears to be an important factor in the initial phase of pulmonary vascular remodeling.

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