Chronic Hypoxia- and Monocrotaline-Induced Elevation of Hypoxia-Inducible Factor-1α Levels and Pulmonary Hypertension

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Key Words
Chronic hypoxia • Monocrotaline • Hypoxia-inducible factor • Pulmonary hypertension

Abstract
A close relationship exists between hypoxia-inducible factor (HIF)-1α and pulmonary hypertension. The present study was carried out to explore if there are temporal alterations in HIF-1α levels during prolonged hypoxia and after monocrotaline (MCT) treatment. First, young Wistar rats were divided into 5 groups: control, hypoxia-1, hypoxia-2, hypoxia-3 and hypoxia-4. Hypoxic rats were placed in a closed hypobaric chamber (380 mm Hg) for a 1-week (hypoxia-1), 2-week (hypoxia-2), 3-week (hypoxia-3) or 5-week (hypoxia-4) period. Second, other young Wistar rats were divided into 4 groups: control, MCT-1, MCT-2 and MCT-3. MCT-treated rats were injected subcutaneously once with MCT (60 mg/kg) for a 1-week (MCT-1), 2-week (MCT-2) or 3-week (MCT-3) period. Subsequently, pulmonary arterial pressure (Ppa) and the weight ratio of the right ventricle to the left ventricle plus the septum [RV/(LV + S)] were measured, and lungs were obtained for the determination of HIF-1α via Western blot analysis. Both hypoxia and MCT induced temporal increases in the Ppa, the ratio RV/(LV + S) and HIF-1α levels. A close relationship between the Ppa and HIF-1α level was found in both hypoxia- and MCT-treated animals. In addition, the PaO₂ level significantly decreased in rats 1–3 weeks after MCT treatment. These results, along with previous data in the literature, suggest that both chronic hypoxia- and MCT-induced lung hypoxia activate an increase in the production of HIF-1α, and result in vascular remodeling and pulmonary hypertension.

Introduction

Cellular responses to chronic hypoxia involve changes in gene expression that are mediated by the transcriptional regulator hypoxia-inducible factor 1 (HIF-1). HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β subunits [15, 17]. The biological activity of HIF-1 is determined by the expression and activity of the HIF-1α subunit [15]. HIF-1α plays a very general role by signaling the existence of hypoxia to the transcriptional machinery in the nuclei of all cells [15].

Studies on HIF-1α during chronic hypoxia are rare. A temporal relationship between HIF-1α levels and the duration of chronic hypoxia was found in the brain of rats [2]. HIF-1α accumulated rapidly during the onset of hypoxia and did not fall for 14 days but fell to normal by
21 days despite the continuous low arterial O2 tension. In the lungs, on the contrary, HIF-1α mRNA levels increased 2.2-fold, suggesting increased HIF-1α levels, after 3 weeks of chronic hypoxia [11]. However, the nature of the trend in the temporal increase in HIF-1α in the lungs during continuous hypoxia is still not clear.

A close relationship exists between HIF-1α and pulmonary hypertension. Yu et al. [19] demonstrated that partial loss of HIF-1α expression, i.e., being heterozygous for the null allele (Hif1α+/−), significantly delayed development of polycythemia, right ventricular hypertrophy, pulmonary hypertension and pulmonary vascular remodeling during chronic (6-week) hypoxia. However, the temporal relationship between HIF-1α expression and the development of pulmonary hypertension during prolonged hypoxia is not clear. In addition, we previously showed that monocrotaline (MCT)-treated rats developed hypoxemia 2–3 weeks after MCT treatment. It is not clear whether this type of hypoxemia following MCT treatment also triggers an increase in HIF-1α levels. Although somewhat different, both chronic hypoxia [6, 8] and MCT [9] cause a temporal trend of development of pulmonary hypertension.

The present study was carried out to explore if there are temporal alterations in HIF-1α levels during prolonged hypoxia and after MCT treatment. Then, we examined whether the temporal progression in chronic hypoxia- and MCT-induced pulmonary hypertension is related to the altered HIF-1α levels. In addition, MCT-induced hypoxemia was also investigated.

Materials and Methods

Animal Preparation

This study was divided into two parts. In the first part, 35 7-week-old Wistar rats weighing 240 ± 5 g were evenly divided into 5 groups: control, hypoxia-1, hypoxia-2, hypoxia-3 and hypoxia-4. Animals in the control group were placed in a room with a hypobaric chamber but breathed room air (at 750 mm Hg). Animals in the hypoxic groups were placed in a closed hypobaric chamber with a barometric pressure of 380 mm Hg for a 1-week (hypoxia-1), 2-week (hypoxia-2), 3-week (hypoxia-3) or 5-week (hypoxia-4) period. Animals were exposed to hypoxic conditions from 17.00 to 8.00 h the following day (intermittent exposure) and to room air the rest of the time.

In the second part of the study, 32 7-week-old Wistar rats weighing 246 ± 6 g were evenly divided into 4 groups: control, MCT-1, MCT-2 and MCT-3. Animals in the control group were subcutaneously injected with saline. Rats in the MCT-treated groups were subcutaneously injected once with MCT (60 mg/kg), and the functional study was carried out for a 1-week (MCT-1), 2-week (MCT-2) or 3-week (MCT-3) period following MCT treatment.

Determinations of Pulmonary Arterial Pressure and Right Ventricular Hypertrophy

Both pulmonary arterial pressure (Ppa) and right ventricular hypothyphy were determined according to our previous method [4]. Briefly, the chest of an anesthetized-ventilated rat was opened via a midline incision. A 22-gauge needle filled with heparinized saline was inserted through the wall of the right ventricle and advanced into the pulmonary artery to measure its pressure. For MCT-treated rats and their control group, blood was sampled from the carotid artery and placed on ice for determination of pH and blood gases within 2 h. The heart was then excised; the weights of the right ventricle (RV) and left ventricle plus the septum (LV + S) were separately obtained. The weight ratio RV/(LV + S) was used as an index of right heart hypothyphy. Also, lung tissue was collected for analysis of HIF-1α.

Western Blot Analysis of HIF-1α

Lung tissue (approximately 15 mg) was homogenized and centrifuged, and protein concentrations of the lung tissue extract were determined by the Bradford protein assay (Bio-Rad). Ten microliters of the tissue extract containing 20 μg of protein was added to a 10-μl mixture of loading buffer (98%) with 2-mercaptoethanol (2%). The above mixture was boiled at 95°C for 5 min and then cooled and stored at −70°C for later analysis within 1 week. For SDS-PAGE, 10 μl of the above sample (1 μg protein/μl) and standard protein markers (Amersham Biosciences, Piscataway, N.J., USA) were first loaded onto the stacking gel, and then 30 V and 15 mA were applied. After all proteins had reached the bottom of the stacking gel, 100 V and 48 mA were applied to separate proteins in the separating gel. After electrophoretic transfer onto a PVDF membrane (Amersham Biosciences) which was preimmersed in 100% methanol and then transfer buffer, each membrane was washed with washing buffer. After 3 washes in washing buffer, membranes were blocked with 3% BSA for 1.5 h at room temperature. Subsequently, the PVDF membranes were incubated with monoclonal anti-HIF-1α IgG antibody (Novus Biologicals, Littleton, Colo., USA) in washing buffer overnight at 4°C. Primary conjugates were visualized on the PVDF membrane with an anti-mouse IgG-biotinylated antibody coupled with streptavidin-horseradish peroxidase-enhanced chemiluminescence (Pierce, Rockford, Ill., USA). The PVDF membrane was placed to a film negative, and the film was developed in a darkroom. The density of protein was then read with the ImageMaster TotalLab program (Amersham Biosciences).

Measurement of pH and Blood Gases

The syringe containing the whole blood was sealed with a cap, and pH and blood gases were measured using a Stat Profile 9 analyzer (NOVA, Waltham, Mass., USA).

Statistical Analysis

All data are reported as the mean ± SEM. One-way analysis of variance was used to evaluate differences among groups. If a significant difference existed among groups, the Newman-Keuls test was used to differentiate differences between any two groups. A linear regression analysis was used to evaluate the correlation between HIF-1α levels and Ppa, as well as between HIF-1α levels and the weight ratio RV/(LV + S). The R value obtained with the regression was examined by the F test to establish the significance of the regression. A difference was considered significant when p < 0.05.