CO Inhalation at Dose Corresponding to Tobacco Smoke Worsens Cardiac Remodeling after Experimental Myocardial Infarction in Rats

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We hypothesized that inhalation of carbon monoxide (CO) (500 ppm), similar to that in tobacco smoke, disturbs the cardiovascular adaptation after myocardial infarction by increasing remodeling. Four groups of rats were assessed. Two groups had myocardial infarction induced by the ligation of the left coronary artery: the first group was exposed to air (infarcted air group, n = 12), and the second was exposed to CO (infarcted CO group, n = 11). They were compared to two sham-operated groups, a control air group (n = 10), and a control CO group (n = 7) exposed (3 weeks) to CO. Aerobic endurance capacity was assessed in both the infarct CO and infarct air group (endurance capacity = 0.043 ± 0.006 m.min−1.g−1 vs. 0.042 ± 0.005 m.min−1.g−1, not significant). In the infarcted CO group compared to the infarcted air group, the dilatation of the left ventricle observed 3 weeks after infarction was increased, (left ventricular diastolic (LVD) diameter (D) = 9 ± 0.4 vs. 7 ± 0.4 mm, p < 0.05; right ventricular diastolic pressure (RVD) diameter (D) = 6 ± 0.6 vs. 4.1 ± 0.4, p < 0.05), and the diastolic posterior wall thickness was augmented (posterior wall diastolic thickness = 1.7 ± 0.1 vs. 1.3 ± 0.1 mm, p < 0.05). Hemodynamic pressure measurements in both ventricles and pulmonary artery showed elevated diastolic pressure after CO exposure compared to air exposure (LVD pressure = 32 ± 1.6 vs. 19 ± 2.3 mm Hg, p < 0.05; right ventricular diastolic pressure = 16 ± 1.6 vs. 8.6 ± 1.6 mm Hg, p < 0.05; pulmonary arterial pressure in diastole (PAD) = 27 ± 1.6 vs. 20 ± 2.3 mm Hg, p < 0.05). In the infarcted CO group, the infarct size increased. Echocardiography and histology showed hypertrophy of the contralateral wall similar to that observed in the noninfarcted control CO group. In conclusion, chronic CO inhalation worsens heart failure in rats with myocardial infarction by an increase in the infarct size and hypertrophy remodeling.

Key Words: remodeling; heart failure; infarction.

The toxic effects of high doses of carbon monoxide have been well documented. CO and particles emitted by tobacco consumption after myocardial infarction are positively correlated with an adverse outcome (Wellenius et al., 2004). The gas component of cigarette smoke contains 2 to 6% CO; smokers inhale concentrations as high as 400 ppm and have elevated carboxyhemoglobin levels (Coburn et al., 1965). CO is considered to be a toxic pollutant and poisons by binding the iron-containing heme group of hemoglobin and other enzymes (Ernst and Zibrak, 1998; Villamor et al., 2000), resulting in hypoxemia. Effect of tobacco smoke on myocardial infarct size increase has been well demonstrated in rats (Wellenius et al., 2004; Zhu et al., 1994). CO also induces cardiac hypertrophy, predominantly in the left ventricle by an unknown mechanism (Penney et al., 1984). Surprisingly, no previous study focused on the effects of CO on cardiac remodeling after myocardial infarction. Remodeling includes hypertrophy of the cardiomyocytes, growth of the capillary network, and an increase in interstitial collagen into the noninfarcted myocardium. These compensatory mechanisms may be beneficial early after infarction, but may have adverse effects when activated for a long time (Cleutjens et al., 1999). The impact of CO on post infarct remodeling might be an important issue for recommendation of tobacco stopping immediately after infarction.

We hypothesized that chronic exogenous CO inhalation might disturb the cardiac remodeling and have an impact on cardiac function in rats with experimental myocardial infarction.

MATERIAL AND METHODS

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Animal model. Male Wistar rats (Harlan, France) weighing ~320 g were anesthetized with halothane (2%), a volatile anesthetic administered initially with a mask aerated with a mixture of oxygen and air. This method allows a convenient control of the duration of anesthesia, since the animal remains anesthetized while it breathes halothane. Moreover, the animal wakes up few minutes after the surgery with a short wake-up post anesthetic phase and a remarkable decrease in the mortality. Under anesthesia, rats were intubated and ventilated with a mixture of halothane (2%) and oxygen and air, using...
a Palmer 2345 ventilator. A left thoracotomy was performed with positive pressure ventilation. The heart was rapidly exteriorized, and the left coronary artery was ligated on its proximal segments, with 6-0 silk sutures. After 2–3 min, part of the left ventricle became white, with reduced motion, confirming the success of the ligation in preventing blood perfusion of the myocardium. After replacing the heart in the thorax, the lungs were inflated by increasing the positive end-expiratory pressure; the muscle layer and skin were then closed separately. The animals woke up a few minutes after the ventilation was ceased and were extubated immediately. The rats were subsequently returned to their cages, monitored as normal, and allowed food and water ad libitum for 1 week before being placed in the exposure chamber.

Of the four groups of rats assessed, two groups had myocardial infarction. The first had ligation of the left coronary artery and were exposed to air (infarcted air group, n = 12), and the second group had ligation of the left coronary artery and were exposed to CO (infarcted CO group, n = 11). The control group (n = 10) of sham-operated rats without the ligation of the left coronary artery were exposed to air (control air group) and the final group (n = 7) was sham-operated without the ligation of the left coronary artery and exposed to CO (control CO group). After one week of recovery from the surgical procedure, the two groups of rats to be exposed to CO were placed in an exposure chamber inflated with an air-CO (500 ppm) mixture for 21 days and maintained at 24°C. The two air groups were placed in same exposure chamber inflated with air for 21 days at 24°C. The CO concentration in the exposure chamber was continuously monitored (Analyzer surveyor).

**Exposure protocol.** Briefly, simulated environments were obtained using four similar chambers (one per group) fitted with clear plastic glass doors for illuminating and viewing the animals. The chamber were aerated using a specific vacuum pump (Becker Mot633, Rambouillet, France); air entered the system via a filter and flowed through each chamber at 100 l/h. CO conditions were obtained using a 9-m³ steel high pressure CO tank (Air Liquide, Paris, France) delivering a 99.98% CO mixture. CO was introduced into the air-intake side of the chamber via a CO mass flow controller system (Aalborg GFC G19149, New York, USA) and secured electrovalves; chamber atmospheres were mixed using electric fans. Atmospheric pressure, CO (500 ppm), and temperature conditions were continuously monitored using calibrated sensors. The two control groups (control air and control CO) were maintained in normoxic conditions (PIO₂ = 159 mm Hg) with or without CO in two of these chambers, while infarcted groups (infarcted air and infarcted CO) were exposed in the two remaining chambers. Using heparinized glass microtubes, blood samples were withdrawn by puncture in the retro-orbital sinus vein at the end of exposure. The blood samples were stored in eppendorf tubes containing EDTA and analyzed for carboxyhemoglobin (COHB) levels and hematocrit control (Bayer M845, Paris, France) in all groups. All animals were fed ad libitum with free access to tap water. Simulated environments were interrupted three times a week for <5 min to provide food and water to the animals. Room temperature was maintained at –21°C using air conditioning (Starclima Oasi Moorea, Paris, France) for each group, and three animals were kept in each cage on a 12:12 h light-dark cycle at the same time.

**Echocardiography.** An echocardiogram was performed the day before the 3-week protocol exposure to CO or air in the chamber and 2 days after exposure. Echocardiography required anesthesia of about 30 min and the minimum cardiac negative inotropic effects possible. For this reason we chose ketamine (50 mg/kg) and xylazine (0.5 mg/kg) ip. Halothane was not used during surgery due to an observed secondary tachycardia. The right carotid artery and jugular vein were cannulated with a polyethylene glycol catheter, which was connected to a Baxter inflow transducer and a Hewlett Packard 78342A recorder. The right carotid artery catheter was advanced into the left ventricle, while the right jugular vein catheter was introduced into the pulmonary artery via the right atrium and right ventricle, in order to obtain end-diastolic and end-systolic pressures.

**Hemodynamic measurements.** Hemodynamic measurements were made after the treadmill exercise and echocardiogram, on Day 3 after the 3-week protocol. Hemodynamic measurements require deeper anesthesia, and for this reason we chose to anesthetize the rats with ketamine (50 mg/kg) and chlorpromazine (0.5 mg/kg) ip. Halothane was not used during surgery due to an observed secondary tachycardia. The right carotid artery and jugular vein were cannulated with a polyethylene glycol catheter, which was connected to a Baxter inflow transducer and a Hewlett Packard 78342A recorder. The right carotid artery catheter was advanced into the left ventricle, while the right jugular vein catheter was introduced into the pulmonary artery via the right atrium and right ventricle, in order to obtain end-diastolic and end-systolic pressures.

**Cardiac morphometry.** After the hemodynamic measurement at the end of the exposure protocol, all the anesthetized rats from infarcted groups (infarcted air group, n = 12, and infarcted CO group, n = 11) were killed by exsanguination. The hearts were quickly removed and trimmed of pericardium, visible fat, and blood vessels. The heart was placed in a bath containing physiological saline solution to keep the heart humid and in the best rounded shape. The heart was positioned with a needle under a camera CCD connected to a microscope with a ×10 objective. The images of the heart and the area of the infarcted part were filmed and stored for further analysis. After the images-recording procedure, the right ventricle wall and left ventricle plus septa were carefully dissected. Left ventricle was rinsed. LV + S and RV were blotted dry and weighed without fixation on a precise analytical scale (Sartorius BP 160P). The infarcted area was dissected and separately weighed. For images analysis, we used analysis software (Optimas, Imasys, France). We estimated the surface of the fibrotic zone and the maximal surface of the heart on three different images. The surface of the fibrotic zone was delimited by the macroscopic differences (color). The surface of the heart was estimated on images with the maximal size to avoid bias due to the position of the heart during the recording procedures of the images. After the surface procedure recording, the hearts were placed in formalin for further histological analysis (see below).

**HO-1 expression.** HO-1 expression was assayed using immunostaining. Following the hemodynamic measurement on Day 3 after the end of the exposure protocol, the noninfarcted groups of rats (control air group, n = 10, and control CO group, n = 7) were killed by exsanguination. The hearts were quickly removed, and trimmed of pericardium, visible fat, and blood vessels. For the infarcted air and infarcted CO groups, a separate series of six rats were assessed. The hearts from all four groups were dissected for histological examination. Cardiac tissue was embedded in paraffin, and 7-μm thick sections taken. Slices were then stained with hemalin-eosin-safran, a trichrome technique that used safran, which specifically stained the fibrotic zone. After deparaffinization and rehydration, slices were incubated with a primary antibody, (rabbit polyclonal anti-rat HO-1, Interchim, France) at 4°C for 12 h. The antigen-antibody reaction was detected using a molecular probe Alexa fluor dye goat anti-rabbit secondary antibody (Interchim, France). The positive reaction was visualized by fluorescent microscopy.

**Statistics.** Results are expressed as mean ± standard error (SEM). Statistical analysis was made with two way ANOVA tests. Differences were considered significant when p < 0.05.
RESULTS

Congestive Heart Failure in Rats with Myocardial Infarction (MI)

The infarct air group was compared to the sham-operated control air group. Immediately following coronary artery ligation, all rats showed a typical increase from 15 ± 4 ms to 28 ± 5 ms in the QRS complex recorded in the conventional leads (I, II, III–VR, VL, VF). Three weeks after the experimental surgery, the infarcted rats showed an increase in the P-R interval from 38 ± 10 to 52 ± 9 ms (p < 0.05). The endurance capacity was lower in the MI group when compared to the sham group (Fig. 1). Echocardiography showed anterior akinesia corresponding to the infarct area. Both diastolic and systolic left ventricular diameters were significantly increased in the infarct air group when compared to the control air group. Immediately following coronary artery ligation, all rats showed a typical increase from 15 ± 4 ms to 28 ± 5 ms in the QRS complex recorded in the conventional leads (I, II, III–VR, VL, VF). Three weeks after the experimental surgery, the infarcted rats showed an increase in the P-R interval from 38 ± 10 to 52 ± 9 ms (p < 0.05). The endurance capacity was lower in the MI group when compared to the sham group (Fig. 1). Echocardiography showed anterior akinesia corresponding to the infarct area. Both diastolic and systolic left ventricular diameters were significantly increased in the infarct air group when compared to the control air group (respectively, in mm, 7.25 ± 0.22 vs. 5.77 ± 0.07; p < 0.001 and 4.46 ± 0.20 vs. 2.94 ± 0.08; p < 0.001). The fractional shortness was significantly (p < 0.001) decreased in the rats with MI (48.9 ± 4.7%) compared to control group (38.5 ± 4.85). No changes were found in the anterior and the posterior diastolic wall thickness (Table 1). The posterior wall systolic thickness was unchanged, whereas the anterior wall systolic thickness was reduced, corresponding to infarct area (Table 1).

In these experiments, the rats with MI had a marked increase in the end-diastolic left and right ventricular pressure, and there was a twofold increase in mean pulmonary artery pressure (Table 2).

Effect of CO on Cardiac Functions Following MI

During exposure, the weight increase was similar between all four groups. The mean weight of the rats after the 3-week protocol was similar in the infarct air group and the infarct CO group (416 ± 64 g vs. 414 ± 51 g, not significant [NS]). After exposure, we measured the hematocrit (Ht) and carboxyhemoglobin (COHb) in these two groups. Following infarct, the hematocrit was significantly (p < 0.05) augmented in the CO group (57.4 ± 1.8%) compared to those animals receiving air (40.1 ± 0.5%). Similarly, COHb was elevated in animals exposed to CO (29.8 ± 1.8% vs. 2.1 ± 0.4%; p < 0.05). No significant change in the ECG was observed in the infarct CO group compared to the infarct air group. The resting heart rate, respectively, in the infarct air and in the infarct CO groups,

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Air control</th>
<th>Air infarct</th>
<th>Infarcted CO</th>
<th>Control CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDD</td>
<td>5.77 ± 0.07</td>
<td>7.25 ± 0.22</td>
<td>9 ± 0.04</td>
<td>5.25 ± 0.12</td>
</tr>
<tr>
<td>LVSD</td>
<td>2.94 ± 0.08</td>
<td>4.46 ± 0.20</td>
<td>6 ± 0.08</td>
<td>2.46 ± 0.10</td>
</tr>
<tr>
<td>PWDT</td>
<td>1.40 ± 0.04</td>
<td>1.42 ± 0.05</td>
<td>1.7 ± 0.01</td>
<td>2.64 ± 0.10</td>
</tr>
<tr>
<td>PWST</td>
<td>2.43 ± 0.11</td>
<td>2.21 ± 0.08</td>
<td>2.1 ± 0.3</td>
<td>3.29 ± 0.18</td>
</tr>
<tr>
<td>AWDT</td>
<td>1.25 ± 0.04</td>
<td>1.28 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>2.7 ± 0.10</td>
</tr>
<tr>
<td>AWST</td>
<td>2.60 ± 0.06</td>
<td>2.18 ± 0.09</td>
<td>2.1 ± 0.2</td>
<td>4.14 ± 0.24</td>
</tr>
</tbody>
</table>

Note. LVDD: left ventricle diameter end diastolic; LVSD: left ventricle diameter end systolic; PWDT: posterior wall diastolic thickness; PWST: posterior wall systolic thickness; AWDT: anterior wall diastolic thickness; AWST: anterior wall systolic thickness.

TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Air control</th>
<th>Air Infarct</th>
<th>Infarcted CO</th>
<th>Control CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDP</td>
<td>4.5 ± 0.6</td>
<td>19 ± 2.3</td>
<td>32 ± 1.6</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>LVSP</td>
<td>102 ± 7.6</td>
<td>67 ± 2.3</td>
<td>87 ± 3.6</td>
<td>108 ± 5.6</td>
</tr>
<tr>
<td>RVDP</td>
<td>1.8 ± 0.6</td>
<td>8.6 ± 1.6</td>
<td>16 ± 1.6</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td>RVSP</td>
<td>16 ± 0.6</td>
<td>34 ± 2.6</td>
<td>38 ± 1.0</td>
<td>15 ± 0.4</td>
</tr>
<tr>
<td>PAPD</td>
<td>11 ± 0.4</td>
<td>20 ± 2.3</td>
<td>27 ± 1.6</td>
<td>10 ± 0.5</td>
</tr>
<tr>
<td>PAP</td>
<td>15 ± 0.4</td>
<td>28 ± 2.6</td>
<td>36 ± 1.3</td>
<td>14 ± 0.3</td>
</tr>
<tr>
<td>PAMP</td>
<td>13 ± 0.4</td>
<td>22 ± 3.0</td>
<td>30 ± 1.3</td>
<td>11 ± 0.3</td>
</tr>
<tr>
<td>CDP</td>
<td>106 ± 5.6</td>
<td>89 ± 2.3</td>
<td>106 ± 4.0</td>
<td>104 ± 4.5</td>
</tr>
<tr>
<td>CSP</td>
<td>139 ± 7.6</td>
<td>103 ± 2.3</td>
<td>122 ± 4.0</td>
<td>129 ± 676</td>
</tr>
<tr>
<td>BMP</td>
<td>116 ± 2.6</td>
<td>94 ± 2.0</td>
<td>111 ± 4.0</td>
<td>121 ± 3.6</td>
</tr>
</tbody>
</table>

Note. Comparative table of the pressure measurements obtained from the control group in air and CO and in the infarct group in air and in air + CO. LVDP: left ventricle diastolic pressure; LVSP: left ventricle systolic pressure; RVDP: right ventricle diastolic pressure; RVSP: right ventricle systolic pressure; PAPD: pulmonary artery diastolic pressure; PAP: pulmonary artery systolic pressure; PAMP: pulmonary artery mean pressure; CDP: carotid diastolic pressure; CSP: carotid systolic pressure; BMP: carotid mean pressure.

∗Differences statistically significant compared to control group.

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was 347 ± 52 versus 321 ± 38 beat per min (NS); P-R interval was 49 ± 1 versus 52 ± 9 ms (NS); QRS enlargement was 18 ± 7 versus 20 ± 6 ms (NS); and vertical electrical axis was 15 ± 8° versus 18 ± 7° (NS). The heart rate decreased by 10% during exposure in the four series of rats, but the difference was not statistically significant.

Aerobic performance was similarly affected in the infarct CO group and the infarcted air group (endurance capacity = 0.043 ± 0.006 m.min⁻¹.g⁻¹ vs. 0.042 ± 0.005 m.min⁻¹.g⁻¹; NS).

Echocardiography showed that the dilation of the left ventricle in the infarcted CO group was greater than that observed in the infarcted air group (9 ± 0.4 vs. 7 ± 0.4; p < 0.05), but the SF was similar (36.8 ± 4.2% vs. 33.1 ± 5.3%; p < 0.01). The posterior wall diastolic thickness was increased in the infarcted CO group (Table 1 and Fig. 2).

Except for the right ventricular systolic pressure, all the hemodynamic pressure measurement values were higher after CO exposure compared to air. (Table 2 and Fig. 3). Systemic pressures evaluated at the carotid level were significantly higher in the infarct CO group when compared to the infarct air group. Exposure to CO in the infarct group induced an average increase in the left and right intraventricular pressures and mean pulmonary pressure of around 30% (Table 2). Moreover, increases in the diastolic left (19 ± 2.3 (air) to 32 ± 1.6 (CO) mm Hg, 40 ± 5%) and right ventricular pressures (8.6 ± 1.6 (air) vs. 16 ± 1.6 (CO) mm Hg, 50 ± 6%) in the infarct CO group were more pronounced than the observed elevation in the carotid systemic pressure (103 ± 2.3 (air) 122 ± 4 (CO) mm Hg) (19 ± 3%). Since the diastolic pressure more precisely evaluated the cardiac failure, this suggests a worsening of heart failure in the infarct CO group compared to the infarct air group.

The weight of the fibrotic tissue was increased in the infarct CO group compared to the infarct air group (Table 3). The surface of the scarred tissue evaluated by macroscopic analysis was also more extensive in the infarct CO group (44 ± 6% vs. 22 ± 5%; p < 0.05) (Fig. 4). The left and the right ventricular weights were increased. Histology confirmed the hypertrophy of the contralateral posterior wall, secondary to cardiomyocytes hypertrophy, without particular fibrosis or collagen increase (Fig. 5).

Hypertrophy was also observed in the control CO group. In this group the hematocrit was 59.9 ± 2.2% and carboxyhemoglobin was 26.5 ± 2.3% (statistically not different when compared to the infarct CO group). The ventricular weight expressed in mg per g of body weight was 3.24 ± 0.1 in the control air group and increased to 4.2 ± 0.11 in the CO control group (p < 0.05). However, the LV to RV ratio did not change (3.34 ± 0.09 vs. 3.75 ± 0.07, NS). This hypertrophy did not induce heart failure, since echocardiography measurements did not show significant changes in shortening fraction (52 ± 7% in

TABLE 3

<table>
<thead>
<tr>
<th>Effects of CO exposure on cardiac anatomical parameters</th>
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<tbody>
<tr>
<td>Infarcted air group</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>n = 12</td>
</tr>
<tr>
<td>LV/BW %</td>
</tr>
<tr>
<td>RV/BW %</td>
</tr>
<tr>
<td>VD/VG %</td>
</tr>
<tr>
<td>Heart/BW %</td>
</tr>
<tr>
<td>Infarct scare/LV %</td>
</tr>
</tbody>
</table>


* Differences statistically significant compared to infarcted air group (p < 0.05).
control CO group compared to 48 ± 6% in control air group) or diastolic diameter (Table 1).

Immunostaining of HO-1 expression on the tissue showed positive labeling in the infarct border. The hypertrophic zone was located in the opposite wall, a distance from the infarct area (posterior wall) and represented the main remodeling phenomenon. In this hypertrophic zone, we observed positive HO-1 expression. In the rats exclusively exposed to CO without infarct (CO control group), HO-1 expression was also shown on the whole myocardium. In contrast, no positive labeling was observed in the groups that were not exposed to CO (control air group; infarct air group), even in the infarct area (Fig. 6).

**DISCUSSION**

Heart Failure in Rat with Myocardial Infarction

The deleterious effect of an infarct is dependent upon its size. This problem led us to evaluate the infarct size in our model, which was of moderate size. The size of the infarct has been classified by Pfeffer (Pfeffer et al., 1979), expressing the infarct scar as a percentage of the left ventricular circumference calculated by histological techniques. In our study, we chose to weigh the fibrotic tissue of the infarct scar at the end of the experiment and express relative to the left ventricular weight. The infarcted fibrotic tissue represented 7.04 ± 2.6% of the left ventricular weight. It is difficult to draw comparisons with the Pfeffer study, since we did not use the same technique to evaluate the infarct size, however, taking into account that the density of the necrotic tissue is probably lower than normal tissue, it seems likely that the extent of the infarct in our study has been underestimated. Moreover, considering the ventricular dilation observed, we may estimate that the size of the infarct for our model was probably comparable to a moderate infarct described by Pfeffer (Pfeffer et al., 1979).

Our results confirm that, in rats, myocardial infarction induces a cardiac insufficiency and left ventricular remodeling. These results demonstrate that our model presents all the characteristics of congestive heart failure. After 3 weeks, the myocardial infarcted rats showed altered aerobic performances.
Echocardiography showed an increase in the left diastolic (LVDD) and systolic (LVSD) ventricular diameters, a decrease in the anterior wall systolic thickness, and consequently a decrease in the fractional shortening. The hemodynamic results confirmed heart failure with elevation of diastolic pressure in the right and the left ventricles, and in the pulmonary arterial pressure. Moreover, the carotid systolic, diastolic, and mean pressure was decreased, which demonstrated either a global vasodilation of the systemic circulation to compensate the difficulties of the heart to keep a efficient cardiac output, or a decrease of the cardiac output as expected in cardiac insufficiency. These results agreed with those previously published (Musch et al., 1992; Pfeffer et al., 1991; Sjaastad et al., 2000; Tian et al., 1996).

**Effect of CO on Cardiac Functions**

One of the major findings of our work is the increase in the infarct size found after CO exposure. In spite of this, the left ventricular weight increased by 15%, the right ventricle increased by 26%, and the ratio VD/VG was 12% higher. These results suggest that CO exposure damaged part of the myocardial tissues, extending the infarct size, but hypertrophied the other healthy part of myocardium, which explains the increase in the ventricular mass.

The increase in the infarct size could be secondary to the hypoxic hypoxemia occurring with CO exposure. By reducing the oxygen delivery to the cardiomyocytes, hypoxic hypoxemia could potentiate the secondary ischemia occurring after the coronary artery ligation in the border zone of the necrotic myocardium tissue. The level of carboxyhemoglobin (29.8 ± 1.8% in CO group) supported this suggestion. The high value of the hematocrit observed in the CO group (57.4 ± 1.8 vs. 40.1 ± 0.5% in air group) may be associated with an increase in blood viscosity that could alter the regimen of the blood flow and also create additive effects on systemic blood pressure.

It has been demonstrated that CO in the pulmonary artery might act as an antiproliferating agent (Villamor et al., 2000). We cannot exclude a similar effect of CO on the coronary neovascularisation observed after the infarction. CO might then reduce the neovascularisation surrounding the infarcted area of the myocardium and increase the infarct size (Jozkowicz et al., 2003); however, this hypothesis requires further investigation.

The consequence of the augmented infarct size is a worsening of the cardiac failure. We observed an increase in the diastolic left and right ventricle pressure in the infarct CO group but not in the control CO group. If CO induces vasodilation, then a decrease in the left ventricular after-load secondary to this vasodilatation should be expected. Our results do not support such hypothesis, since we found an increase in the systemic pressure, which returns back to that observed in sham rats. This relative increase in the systemic pressure induces an extra workload for the left ventricle, which could contribute to the worsening of the cardiac failure. This increased pressure could be interpreted as a consequence of a vasoconstriction phenomenon and increases in the systemic arterial resistance secondary to the release of vasoconstricting factor such as angiotensin, noradrenaline (Lerch and Montes-suít, 1997; Remme, 1993).

The second major observation is the increase in left ventricular remodeling, since the left ventricular diastolic diameters and the posterior wall diastolic thickness were increased. We have shown a hypertrophic response to CO exposure in the control CO group. A precedent study has described this effect, but the mechanism remained unknown (Lakkisto et al., 2002; Melin et al., 2002). It has already been suggested that HO-1 expression was enhanced by exogenous CO exposure (Carraway et al., 2002). Expression of HO-1 in the CO-group confirmed a direct effect of CO on cardiomyocytes. Recently, increased expression of HO-1 has been demonstrated in response to myocardial infarction in rats (Lakkisto et al., 2002). In our study we did not see HO-1 expression in infarct area, perhaps because our experiment was performed 3 weeks after infarction. In the infarct CO group, expression of HO-1 was present in both noninfarct and infarct area. The presence of HO-1 expression on the hypertrophic wall far away from infarction leads to think that this more pronounced remodeling is not only the consequence of infarct size augmentation but is also linked to a direct effect of CO as already suggested (Carraway et al., 2002). This remodeling could have a negative impact on cardiac adaptation after infarction.

We can conclude that exogenous CO inhalation after cardiac infarction, at levels corresponding to tobacco smoke, worsens cardiac failure of rats with experimental myocardial infarction by both increasing in the infarct size and by ventricular remodeling.

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