The Prevention of Monocrotaline-Induced Right Ventricular Hypertrophy

Ryan Hootable, Ph.D.; Samuel Papana, M.D.; and James Laugharn, B.S.

The pyrrolizidine alkaloid, monocrotaline, is the toxic principle of the plant Crotalaria spectabilis. Monocrotaline has been reported to produce pulmonary hypertension and right ventricular hypertrophy when administered to rats by admixture with their chow, or by subcutaneous injection of the pure alkaloid. The development of pulmonary hypertension is associated with medial hypertrophy of the muscular pulmonary arteries.

We have given monocrotaline to male Wistar rats via their drinking water at a concentration of 20 mg/liter. When monocrotaline is chronically administered in this way to rats of an initial weight of 55 gm, a mild right ventricular hypertrophy appears over a 3-4 week period with no evidence of inflammatory changes in the myocardium or pulmonary blood vessels. By contrast, right ventricular hypertrophy following a single subcutaneous injection of monocrotaline has been found by others to be associated with myocarditis and necrotizing arteritis affecting up to 50% of the animals. This report describes some of the characteristics of right ventricular hypertrophy produced by chronic administration of monocrotaline and its prevention by the β-adrenergic antagonist propranolol.

RESULTS

The microscopic appearance of muscular pulmonary arteries in control animals and animals which had received monocrotaline for 21 days in their drinking water is exemplified in Figure 1. The medial thickening and decreased lumen diameter in the monocrotaline-treated animal are readily apparent.

Along with the alterations in the small pulmonary arteries, the right ventricle to body weight ratio increased steadily, reaching twice the control value after a month of monocrotaline intake (Fig. 2). There was no alteration in left ventricular weights. The wet and dry weights of the lung also increased significantly. After 12 days of monocrotaline treatment, the wet lung to body weight ratio changed from $6 \times 10^{-3}$ to $8.5 \times 10^{-3}$ ($P < .001$).

The rate of protein synthesis in the right ventricle, as measured by the incorporation of [1H]leucine, paralleled ventricular to body weight ratio, being significantly elevated from the 12th day of treatment (Fig 3). Acid soluble radioactivity, representing free [1H]leucine, was significantly elevated on the 9th day only. Throughout the period of monocrotaline treatment, no

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Fig. 1. Small pulmonary arteries, stained with elastic van Gieson stain (original magnification 1100 x). A (upper). From a control male Wistar rat. B (lower). From a Wistar rat exposed to monocrotaline (20 mg/liter drinking water) for 3 weeks, illustrating medial thickening. Medial thickening increased progressively with time from this point.

Fig. 2. Ventricle to body weight ratio after monocrotaline treatment. Experimental animals were maintained in a laminar flow hood and were given 20 mg monocrotaline per liter drinking water for the indicated period. Male Wistar rats (Simonsen, California) with an initial weight of 55 ± 5 (range) gm were used for all experiments in this paper. A Left ventricle; B right ventricle. The free walls of the ventricles were dissected for weighing; the septum was excluded. Open symbols were monocrotaline treated. Each point represents the means of 4 animals ± SEM. * P < .05 (Student t test).

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alterations in protein synthesis were seen in the left ventricle. An indication of RNA synthesis was obtained by measurement of \[^{3}H\] orotic acid incorporation into the acid precipitable fraction following administration in vivo. No alterations in incorporation per gram tissue could be detected in the right ventricle over the course of monocrotaline treatment. However, incorporation into the pulmonary arterial trunk doubled over the period of treatment, as shown in Figure 3. Incorporation of orotic acid after 2 or 4 days of monocrotaline was significantly lower than in animals treated for longer periods.

Throughout the period of monocrotaline treatment, no alterations in protein synthesis were seen in the left ventricle.

The development of right ventricular hypertrophy that occurred over the period two- to four weeks after the first exposure to monocrotaline was prevented by the concomitant chronic administration of DL-propranolol (400 mg/liter of drinking water). As shown on Figure 4, rats not given propranolol exhibited the typical increase in right ventricular weight after 28 days of monocrotaline.

**Table 1**—Effect of Chronic Propranolol Treatment on Pulmonary Arterial and Right Ventricular Blood Pressures

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary Artery</th>
<th>Right Ventricle</th>
<th>Ventricle/Body Weight</th>
<th>Pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>systolic</td>
<td>diastolic</td>
<td>systolic</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.3 ± 1.9</td>
<td>14.7 ± 1.4</td>
<td>24.4 ± 3.6</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td>Monocrotaline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ propranolol</td>
<td>26.7 ± 2.3</td>
<td>16.2 ± 1.8</td>
<td>28.7 ± 2.7</td>
<td>1.03 ± 0.16</td>
</tr>
<tr>
<td>Monocrotaline</td>
<td>34.7 ± 4.6*</td>
<td>20.4 ± 3.0</td>
<td>42.2 ± 1.8*</td>
<td>1.45 ± 0.06*</td>
</tr>
</tbody>
</table>

Pressures are in mm Hg and pulse in beats/min. Ventricle-to-body weight ratios are expressed \(\times 10^7\). Data are expressed as means ± SEM and were recorded open-chested, prior to sacrifice. Number of animals in each group is shown in parentheses. Animals were exposed to monocrotaline with propranolol, or monocrotaline alone, as described in the text. *P < .05 compared to untreated group; \(\dagger\) P < .05 compared to propranolol group (Student's t test).

**Discussion**

The method of administration of monocrotaline we use has the advantages of producing mild right ventricular hypertrophy without detectable inflammatory changes. The latest period of several days that elapses before hypertensive changes or cardiac enlargement occur is observed after both chronic and acute administration of monocrotaline. This suggests that changes are occurring at the cellular or subcellular level during this period.
period that result eventually in structural alterations such as arterial medial and right ventricular hypertrophy. This concept is reinforced by the finding of a similar delay in time before elevations occur in the rate of synthesis of protein in the right ventricle, and of RNA in the pulmonary trunk. Right ventricular and pulmonary arterial systolic blood pressures are also not increased during the first two weeks of monocrotaline treatment. Other changes in the arterial wall might include early myogenic, neural or hormonal influences predisposing to heightened muscular tone. The effect of DL-propranolol on the response to monocrotaline was examined in view of the $\beta$-adrenergic antagonist shown by the $\beta$-steroiomer. In addition, both stereoisomers have local anesthetic properties, and inhibit calcium uptake by isolated sarcoplasmic reticulum with an ED50 of 10 $\mu$M. Therefore, propranolol decreases adrenergic activity and decreases calcium movement into the cell by a direct and indirect action. Concurrent administration of propranolol prevented cardiac hypertrophy over the period of the experiment. It is unclear, however, whether the protective effect of propranolol in preventing cardiac hypertrophy is exerted on the heart or lungs. Initial examination of samples prepared from the lungs of propranolol-treated rats suggested that medial hypertrophy is not so marked; quantitative determination of medial thickness has not yet been done. Treatment with propranolol also lessened the increase in pulmonary arterial blood pressure caused by monocrotaline.

This observation offers the opportunity to explore at which level propranolol is acting—capillary bed, muscular arteries, or heart—and by which of its several pharmacologic actions. This may provide a degree of insight into the mechanism of monocrotaline toxicity.

REFERENCES

3 Hurtable R: Unpublished observations

Chemoreceptor Effects on Perfusion of Hypoxic and Atelecatic Lung*


Controversy persists concerning the reduction in pulmonary blood flow following acute lobar atelectasis. Most investigators observed decreased blood flow to atelectatic or unilaterally hypoxic lungs. However, some investigators found unaltered blood flow to acutely atelectatic lobes. These unchanged levels of blood flow were associated with moderate systemic hypoxemia, due to a high pulmonary shunt. Evidence that a similar phenomenon may occur during unilateral hypoxia is provided by Himmelstien and colleagues.* One of their 5 subjects, who developed marked systemic hypoxemia (SaO2 = 70%) when 6 percent oxygen was administered to 1 lung, also failed to shift pulmonary blood flow away from that unilaterally hypoxic lung.

The present studies were conducted to see if the level of arterial Po2 itself might influence the perfusion of an acutely collapsed or hypoxic lobe.

METHODS

Mongrel dogs were anesthetized with sodium pentobarbital and respirated with a dual piston respirator and a Carlen tracheal divider. Cardiac output and left pulmonary arterial blood flow were obtained by electromagnetic flow probes implanted via median sternotomy. Pressure data and blood samples were obtained from a Swan-Ganz pulmonary artery catheter, and from a femoral arterial cannula. Blood gas levels were determined by standard electrodes. Data were taken at 10-20 minute intervals according to several experimental designs:

Hypoxic ventilation (left)-6 dogs 1) Both lungs ventilated with room air; 2) both lungs ventilated with oxygen, right lung continues to receive oxygen while; 3) left lung ventilated with 6 percent O2 in N2; 4) left lung ventilated with N2; 5) left lung returned to 6 percent O2 in N2. Then, while the left lung continues to receive 6 percent O2 in N2; 6) right lung ventilated with room air; 7) right lung returned to 100 percent O2; 8) both lungs return to room air.

Hypoxic ventilation (right)-5 dogs The above maneuvers were duplicated except that the left and right lungs were reversed, left for right and right for left. However, the test sequence remained the same.

Hypoxic ventilation (left, sino-aortic denervated)-6 dogs The maneuvers in the first experiment were duplicated in dogs which had undergone sino-aortic denervation of carotid and aortic chemoreceptors and baroreceptors two to three weeks previously.* Atelecatic (left)-6 dogs Steps 1,2,3,6 and 7 of the first experiment were repeated in the same sequence except that instead of ventilating the left lung with 6 percent O2 in N2 (steps 3,6 and 7), the left lung was made acutely atelectatic by denervation (step 2) and then occluding its airway.

Atelecatic (left, sino-aortic denervated)-6 dogs Fourth experiment was conducted on dogs denervated as in third experiment.

RESULTS

Left lung blood flow decreased significantly from control during hypoxic ventilation (Fig 1) or acute atelectasis (Fig 2) of the left lung in both intact and denervated dogs as long as arterial hypoxemia was prevented (P < .01). When mild arterial hypoxemia was induced by room air ventilation of the right lung while the left lung remained collapsed or hypoxic, blood flow to the left lung increased to near control levels in intact animals, but was unaffected in chemodenerovated animals. Signifi-

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