Effect of ligustrazine on pulmonary vascular changes induced by chronic hypoxia in rats

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SUMMARY

1. Acute and chronic effects on the pulmonary circulation of ligustrazine, a chemically identified and synthesized principle of a Chinese herb, were studied in rats. It dilated lung vessels and reversed hypoxic pulmonary vasoconstriction.

2. In rats kept 2 weeks in 10% O₂ in a normobaric chamber and simultaneously treated with ligustrazine, right ventricular hypertrophy and muscularization of pulmonary arterioles were attenuated compared with saline-treated rats. Pulmonary artery pressure, measured in isolated lungs perfused at a constant flow rate, was also less in ligustrazine-treated rats.

3. In isolated blood-perfused lungs of chronically hypoxic and control rats, the relation between pressure and flow was measured during normoxia (ventilation with air plus 5% CO₂), hypoxia (2% O₂ plus 5% CO₂) and after ligustrazine during continued hypoxia. Alveolar pressure was always greater than left atrial pressure; thus flow was determined by the pulmonary artery minus alveolar pressure difference.

4. Pressure/flow lines were measured during normoxia in four groups of rats: (1) control, saline-treated; (2) control, ligustrazine-treated; (3) chronically hypoxic, saline-treated; (4) chronically hypoxic, ligustrazine-treated. Both chronically hypoxic groups had steeper lines (higher resistance) than the control groups, which were similar in all respects. However, in chronically hypoxic rats, the extrapolated intercept of the line on the pressure axis, probably attributable to small newly muscularized arterioles in a state of tor-e, was much increased in the saline-treated group but did not differ from controls in the ligustrazine-treated group.

5. The proportion of muscularized small vessels in the four groups was: control, saline-treated 6.3 ± 1.0%, control, ligustrazine-treated 5.7 ± 0.6% (not significant), chronically hypoxic, saline-treated 27 ± 3.1%, chronically hypoxic, ligustrazine-treated 14.7 ± 2.1% (P < 0.01). We attribute the change in the pressure/flow line in ligustrazine-treated hypoxic rats to this attenuation of new muscle growth.

6. After chronic ligustrazine treatment, its acute effect was undiminished.

Key words: chronic hypoxia, ligustrazine, pulmonary hypertension, pulmonary vasodilatation.

Abbreviations: LV+S, left ventricle plus septum; Ppa, pulmonary artery pressure; P/Q, pressure/flow; Ptr, tracheal pressure; RV, right ventricle; TWPV, thick-walled peripheral vessels.

INTRODUCTION

Pulmonary artery pressure (Ppa) rises in both man and animals exposed to acute or chronic hypoxia. In chronic hypoxia, although hypoxic pulmonary vasoconstriction may still play a part, a large component of the high pressure is due to muscularization and narrowing of normally thin-walled arterioles. Polycythaemia also contributes and there is right ventricular hypertrophy. Hypoxic vasoconstriction may be the initiating cause of the high pressure, but its mechanism still eludes us; there may be release of a mediator or hypoxia may directly affect the smooth muscle cell. Neither do we understand the stimuli which lead to growth of new muscle in small vessels or what causes deposition of new elastin and collagen in larger vessels, although a number of potential growth factors have been discovered.

There is no specific drug comparable in effectiveness to those used in systemic hypertension with which to treat pulmonary hypertension. Drugs are particularly needed...
for so-called primary pulmonary hypertension and for post-operative pulmonary hypertensive crises in children after cardiac surgery. Most so far used either cause systemic hypotension or must be given intravenously. The requirements are for a substance which reverses hypoxic vasoconstriction (when this is a component) and prevents or reverses structural changes; it must also be administered orally and suitable for very prolonged use.

Recently, it was reported [1, 2] that ligustrazine, a chemically identified and synthesized principle of a traditional Chinese herb, could significantly reduce the pulmonary hypertension caused by chronic hypoxia in rats. It also reduced acute hypoxic pulmonary vasoconstriction in isolated perfused lungs of normal rats [3] and in ferret lungs in vivo [4] in a dose-dependent manner. The aim of this work was to determine whether treatment with ligustrazine during exposure of rats to prolonged hypoxia would reduce the pulmonary hypertension, right ventricular hypertrophy and muscularization of arterioles which normally ensue. We also compared the pressure/flow ($P/Q$) relations in these lungs with those of control rats to look for functional consequences of structural changes.

METHODS

Animals and hypoxic chamber

Wistar SPF (Tuck's) rats were obtained at 28 days of age and allowed to adapt to the laboratory for a few days. They were divided into four groups, two kept in the hypoxic chamber (chronically hypoxic rats) and two in the same room in air (control rats). The four groups were as follows: (1) control, saline-treated ($n = 5$); (2) control, ligustrazine-treated ($n = 5$); (3) chronic hypoxic, saline-treated ($n = 11$); (4) chronic hypoxic, ligustrazine-treated ($n = 11$).

At the time of entry into the experiment body weight was 140–230 g. All four groups of rats were kept for 2 weeks before tests were made, during which time they received twice daily injections of either ligustrazine (8 mg/100 g) or saline [0.9% (w/v) NaCl, volume and pH as drug] intraperitoneally. Chronically hypoxic rats were kept for 2 weeks in 10% $O_2$ in a normobaric chamber previously described [5]; they were put in at intervals and compared at similar times with controls which had reached the same age. The chamber was opened twice daily for the injections and also for cleaning and replenishing food and water as necessary. Two separate experiments were performed, $n = 20$ in the first, $n = 12$ in the second (chronic hypoxic groups only). As results were very similar, the two have been considered together. Ligustrazine was obtained as ampoules (tetramethylpyrazine, injectio ligustrazini hydrochloridi, Chinese government product, 20 mg/ml). It has the following structural formula:

\[
\begin{array}{c}
\text{H}_3\text{C} \\
\text{H}_2\text{C} \\
\text{N} \\
\text{N} \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{HClH}_2\text{O}
\end{array}
\]

Isolated perfused lung preparation

After pentobarbitone anaesthesia (60 mg/kg intraperitoneally), the isolated rat lungs were perfused in situ with a pump by a method previously described [5]. Blood for the perfusion circuit was taken from one or two normal rats (packed cell volume was therefore normal) and pH was corrected to 7.35–7.45 with sodium bicarbonate. The perfusate was kept at 39°C. Ventilation was with air plus 5% $CO_2$ (normoxia) or 2% $O_2$/5% $CO_2$/balance $N_2$ (hypoxia); end-expiratory pressure was 2–3 mmHg (0.3–0.4 kPa). Blood flow was constant at 20 ml/min except during measurement of $P/Q$ relations. Although growth is retarded in chronically hypoxic rats, growth of the lung is disproportionate and lung vascular volume similar to controls [5]; thus we used the same flow rate for all groups. $P_{pa}$ and tracheal pressure ($P_{tr}$) were measured with electromanometers (Electromed) and displayed on a pen recorder (Advance Bryans C1013). Blood flow was measured with a Robinson electromagnetic flowmeter [6].

Measurement of ($P_{pa}/Q$) relations

$P_{pa}$ and flow were displayed on the two axes of an XY recorder (Bryan). Flow was lowered slowly by altering the perfusion pump speed to give flow rates of 20, 15, 10, 5 and 0 ml/min. The lines were linear except at very low flow rates ($< 5$ ml/min), where they curved convexly to the pressure axis as shown in Fig. 1. The linear part was extrapolated to the pressure axis and the intercept so formed was considered the effective downstream pressure during the linear part of the flow range when flow is $\propto P_{pa} - \text{Int}$.  

![Fig. 1. Diagram showing the shape of $P_{pa}/Q$ lines as measured on an XY recorder. The dotted line extrapolating the linear portion of the line forms an intercept (Int) on the pressure axis. This is the effective downstream pressure during the linear part of the flow range when flow is $\propto P_{pa} - \text{Int}$.](image)
pressure. That is, since \( P_{tr} \) was greater than zero and left atrial pressure was always less than zero, flow was determined over the linear \( P/Q \) range by the difference between \( P_{pa} \) and the extrapolated intercept; the lung was in West's zone 2 [7]. The slope of the \( P/Q \) line was taken as the resistance of the pulmonary arterial system and the intercept as due to small collapsible vessels acting as 'Starling resistors' [8, 9]. The intercepts were due either to alveolar pressure (= \( P_{tr} \)) or to muscular tone in small collapsible vessels as described by Permutt & Riley [9].

**Measurement of heart weights and assessment of muscularization of arterioles**

After completion of the lung perfusion, the hearts were removed and the right ventricle (RV) and left ventricle plus septum (LV+S) were weighed. The lungs were perfused through the tracheal cannula with 10% (v/v) phosphate-buffered formaldehyde at 20 cmH\(_2\)O pressure. After usual processing, a standard transverse piece of the left lung was sectioned and stained by Humberstone's modification of Gomori's method for elastin. The method of counting muscularized arterioles has been previously described [10], but, briefly, consisted of counting all vessels equal to or less than 50 \( \mu \)m in diameter, adjacent to alveoli or alveolar ducts and the percentage of these with a double elastic lamina round at least half their wall. These vessels include small veins which cannot be distinguished from arterioles with certainty without the use of a filling medium. We prefer this semi-quantitative method for looking at numerous lungs because many vessels are examined (ca. 350 vessels/section [5]). In the alternative method where medial thickness is measured, very few vessels are measurable per section and the analogous vessels in controls lack a medial coat to measure [10].

**Statistics**

Mean and \( \text{sem} \) are given in the text and Tables. Only the following comparisons were made using the Student's unpaired \( t \)-test; (1) groups treated with ligustrazine or saline (i.e. the groups kept in air or in 10% \( O_2 \)); (2) the effect within each group of changing from normoxia to hypoxia. \( P<0.05 \) was considered statistically significant.

**RESULTS**

**\( P_{pa} \), ventricular weight and nature of arteriolar walls**

Table 1 shows body weight, initial \( P_{pa} \) during normoxia in the isolated perfused lung preparations at a flow rate of 20 ml/min, ventricular weight and the percentage of thick-walled peripheral vessels (TWPV) in the four groups of rats. There was no significant difference in the initial \( P_{pa} \) in the two control groups. In the chronically hypoxic groups, \( P_{pa} \) was significantly higher in saline-treated than in ligustrazine-treated rats. Thus treatment with ligustrazine prevented the rise in \( P_{pa} \) normally seen in chronic hypoxia [5] and the pressure remained close to that of rats living in air.

Table 1 shows that RV weight was similar in the two control groups. In the chronically hypoxic groups, RV was significantly lighter in the ligustrazine-treated than in saline-treated rats. Thus ligustrazine diminished the right ventricular hypertrophy which normally occurs during chronic hypoxia [10, 11].

The percentages of TWPV for the four groups are also shown in Table 1 and individual values and means are shown in Fig. 2. In control rats there was no significant difference. Both chronically hypoxic groups had higher percentages of TWPV than controls, as shown in previous studies [5, 10, 11]; however, the ligustrazine-treated rats had significantly fewer TWPV than the saline-treated rats. Thus ligustrazine attenuated the muscularization normally caused by chronic hypoxia.

**\( P/Q \) relations during normoxia**

Fig. 3 shows mean \( P/Q \) lines for the four groups measured during normoxia, while Table 2 shows the mean values and \( \text{sem} \) for slope, intercept on the pressure axis and the significance of differences between ligustrazine and saline-treated groups. Slopes and intercepts were similar in the two control groups. In the two chronically hypoxic groups the slopes were steeper and the intercepts were greater than in controls, as previously found [5]. There was no significant difference between the slopes in saline- and ligustrazine-treated chronically hypoxic rats.

![](https://example.com/image1.png)

**Table 1. Effect of ligustrazine in control and chronically hypoxic rats**

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th>Chronically hypoxic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ligustrazine-treated ( (n=5) )</td>
<td>Saline-treated ( (n=5) )</td>
</tr>
<tr>
<td>Mean body wt. (g)</td>
<td>264 ± 15.5</td>
<td>276 ± 13.9</td>
</tr>
<tr>
<td>( P_{pa} ) mmHg</td>
<td>19.6 ± 1.0</td>
<td>19.6 ± 1.8</td>
</tr>
<tr>
<td>( P_{pa} ) kPa</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>RV wt. (mg/100 g body wt.)</td>
<td>61.2 ± 1.4</td>
<td>66.9 ± 3.1</td>
</tr>
<tr>
<td>LV+S wt. (mg/100 g body wt.)</td>
<td>208.7 ± 5.8</td>
<td>198.1 ± 11.1</td>
</tr>
<tr>
<td>TWPV (%)</td>
<td>5.7 ± 0.6</td>
<td>6.3 ± 1.0</td>
</tr>
</tbody>
</table>

*Measured during normoxia in isolated lungs.*
Fig. 2. Percentage of TWPV in the four groups of rats. Abbreviations: CL, control, ligustrazine-treated \((n = 5)\); CS, control, saline-treated \((n = 5)\); CHL, chronically hypoxic, ligustrazine-treated \((n = 11)\); CHS, chronically hypoxic, saline-treated \((n = 11)\). Values for individual rats (*) and mean values for groups (—) are shown.

Groups, but the intercept of the ligustrazine-treated group was much reduced and close to that of the control groups.

**P/Q relations in the four groups during hypoxia and after ligustrazine during hypoxia**

**P/Q lines** for the four groups were measured during normoxia, acute hypoxia and 5 min after ligustrazine (4 mg into the blood reservoir) during continued acute hypoxia. Mean results for slopes and intercepts are given in Table 2. The saline- and ligustrazine-treated control and chronically hypoxic groups are compared and, within each group, the values in normoxia and hypoxia are compared. In control rats, treated either with saline or ligustrazine, acute hypoxia increased both slope and intercept significantly. In both control groups, ligustrazine given during continued acute hypoxia brought slopes and intercepts back close to control values. In chronically hypoxic rats slope and intercept increased significantly only in the saline-treated group. Again, ligustrazine, given during acute hypoxia, brought slopes and intercepts back to normoxic values in both chronically hypoxic groups. Thus ligustrazine treatment altered the properties of the pulmonary vascular bed and reduced the effects of acute hypoxia in the chronically hypoxic groups; the dilator action of acutely administered ligustrazine during acute hypoxia remained undiminished after daily administration for 2 weeks.

**DISCUSSION**

Ligustrazine comes from the root of an umbelliferous plant, *Ligustrazine wallichii* Franch, which grows mainly in the south-west and north regions of China. It was traditionally used for 2000 years as a mixture with other Chinese herbs in patients with cardiovascular disease. The active principle, ligustrazine hydrochloride, was recently chemically identified and synthesized \[12\].

Our measurements of $P_{pa}$ in isolated perfused lungs of chronically hypoxic rats kept in a normobaric chamber for 2 weeks showed that the raised $P_{pa}$ normally found under these conditions was significantly reduced by ligustrazine (Table 1). These results are consistent with measurements of $P_{pa}$ made with a catheter *in vivo* in rats kept for 2 weeks in a hypobaric chamber at a simulated altitude of 5000 m \[1, 2\].

Chronic treatment with ligustrazine during exposure to hypoxia attenuated both the right ventricular hypertrophy and muscularization of arterioles normally caused by chronic hypoxia in this species (Table 1, Fig. 2). It had no noticeable adverse effect. No rats died and body weight was not reduced compared with saline-treated rats exposed to hypoxia.
Table 2. Characteristics of $P/Q$ lines in control and chronically hypoxic rats

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th></th>
<th>Chronic hypoxic rats</th>
<th></th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ligustrazine-treated</td>
<td>Saline-treated</td>
<td></td>
<td>Ligustrazine-treated</td>
<td>Saline-treated</td>
</tr>
<tr>
<td>Slope (mmHg ml⁻¹ min)</td>
<td>0.72±0.05</td>
<td>0.67±0.07</td>
<td>NS</td>
<td>0.99±0.10</td>
<td>1.08±0.10</td>
</tr>
<tr>
<td>Intercept (mmHg)</td>
<td>8.0±0.8</td>
<td>6.8±0.7</td>
<td>NS</td>
<td>8.9±1.0</td>
<td>14.0±1.4</td>
</tr>
<tr>
<td>Slope (mmHg ml⁻¹ min)</td>
<td>1.05±0.04***</td>
<td>1.14±0.05***</td>
<td>NS</td>
<td>1.17±0.05</td>
<td>1.39±0.09**</td>
</tr>
<tr>
<td>Intercept (mmHg)</td>
<td>13.5±1.02**</td>
<td>12.9±1.4**</td>
<td>NS</td>
<td>12.3±2.4</td>
<td>24.6±2.0***</td>
</tr>
<tr>
<td>Slope (mmHg ml⁻¹ min)</td>
<td>0.69±0.05</td>
<td>0.65±0.06</td>
<td>NS</td>
<td>0.97±0.13</td>
<td>1.01±0.07</td>
</tr>
<tr>
<td>Intercept (mmHg)</td>
<td>7.2±0.6</td>
<td>6.8±0.8</td>
<td>NS</td>
<td>7.8±1.3</td>
<td>12.5±1.7</td>
</tr>
</tbody>
</table>

$P/Q$ lines measured during normoxia

Ligustrazine treatment had no effect on the $P/Q$ lines of control rats during normoxia, but in the groups exposed to hypoxia, although the slope was unchanged, the intercept was greatly reduced so that it was not significantly different from that of control rats (Fig. 3). Thus in chronically hypoxic rats it seems that the overall resistance of the vascular bed which we define as the slope of the $P/Q$ line, was not reduced. However, the effective intercept on the pressure axis, was greatly diminished. In these experiments left atrial pressure was always less than $Ptr$ or alveolar pressure; thus downstream pressure is attributable either to compression of small vessels by alveolar pressure or to muscle tone in small collapsible vessels [9]. In a previous study we argued that the increased intercept in chronically hypoxic rats might be attributable to the newly muscularized arterioles in a state of tone [8]. We also found evidence in chronically hypoxic rats that the mean site of hypoxic vasoconstriction had apparently changed from small extra-alveolar arteries to vessels which, at least functionally, were alveolar vessels. We considered that they might be the newly muscular arterioles and that the site of vasoconstriction might thus have moved distally. The reduced intercept in the chronically hypoxic ligustrazine-treated rats might therefore have been caused by lesser muscularization of arterioles, a prediction borne out by the reduced number of TWPV found histologically (Table 1). The fact that the overall resistance of the pulmonary arterial bed was not reduced might be due to the fact that ligustrazine has no effect on the deposition of new collagen and elastin in the walls of larger vessels. This deposition contributes substantially to the raised $Ppa$ of chronically hypoxic rats [13].

$P/Q$ lines during acute hypoxia and after ligustrazine during hypoxia

The effect of acute hypoxia was undiminished in control, ligustrazine-treated rats but was attenuated in chronically hypoxic, ligustrazine-treated rats (Fig. 3, Table 2). However, in all four groups, the dilator effect of ligustrazine during acute hypoxia was similar and brought the $P/Q$ lines back to the normoxic position during continued acute hypoxia. Thus the activity of ligustrazine, assessed in this way, was undiminished by chronic administration.

It is of interest to compare this drug with others which have been used experimentally to reduce the consequences of chronic hypoxia in rats. These include $\beta$-adrenoreceptor agonists, $\alpha$- and $\beta$-adrenoreceptor antagonists, angiotensin converting enzyme inhibitors, $Ca^{2+}$-transport inhibitors, cyclo-oxygenase inhibitors, lipoxygenase inhibitors and substances known to inhibit smooth muscle growth (reviewed in [14]). They have, to different degrees, affected $Ppa$, right ventricular hypertrophy, muscularization of arterioles and polycythaemia, indicating the complexity of the growth changes which take place. Only nifedipine has so far been shown to reverse changes once fully developed. Clinically, treatment with this drug has met with some success in primary pulmonary hypertension. We cannot tell whether ligustrazine will prove equal or superior to these agents. Traditional use of the herb suggests that ligustrazine may be active when given orally. Preliminary tests where ligustrazine was given intravenously in ferrets showed that it reduced $Ppa$ more than it reduced systemic blood pressure [4]; its possible selectivity in this respect is a matter of importance. It has so far only been tried in experimental hypoxic pulmonary hypertension.

Further work is required to see if ligustrazine can reduce the fully developed changes caused by chronic hypoxia. Preliminary clinical trials with this drug have been conducted in China. It remains to be seen whether it will be of use in human pulmonary hypertension.

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