Effects of ligustrazine on the pressure/flow relationship in isolated perfused rat lungs

A. Oddoy*, D. Bee, C. Emery, G. Barer

ABSTRACT: Ligustrazine, the synthesized principle of a Chinese herbal remedy shown previously to be a pulmonary vasodilator, was tested in chronically hypoxic and normal rats. Pressure/flow, (P/Q), relations were measured in isolated perfused lungs during normoxia, hypoxia and after reversal of hypoxic vasoconstriction by increasing doses of ligustrazine.

P/Q lines were linear over a wide range and extrapolation to the pressure axis gave an intercept which was the effective downstream pressure for flow. In chronically hypoxic rats the slope of the line was steeper and the intercept greater than in control rats, which we attributed to newly muscularized arterioles with tone. Hypoxia caused an increase in slope and intercept in both groups but the intercept increase was greater in chronically hypoxic rats. In both groups of rats increasing doses of ligustrazine given during continued hypoxia caused a fan of lines which moved progressively towards the control normoxic line. In chronically hypoxic rats it required only 2 mg of ligustrazine to bring the line back to the normoxic position, whereas in controls it required 4 mg. In chronically hypoxic rats the change in intercept with every dose was greater than in control rats; this suggests that ligustrazine mainly relaxes the muscle of small collapsible vessels.

The action of ligustrazine remained in both control and chronically hypoxic rats after administration of an arginine analogue which blocks synthesis of the endothelial relaxant factor nitric oxide. This and previous evidence suggest that ligustrazine is a non-endothelial-dependent pulmonary vasodilator.

A potent, specific pulmonary vasodilator substance which could be used in severe progressive pulmonary hypertension and in post-operative pulmonary hypertensive crises is still lacking. Recently, experimental evidence in animals has shown that ligustrazine (tetramethyl pyrazine HCl), semi-synthesized principle of an ancient Chinese herbal remedy [1], is a potent and possibly relatively specific pulmonary vasodilator. The herb has been used orally with other herbs for "heart disease" for thousands of years. In isolated rat lungs it proved a powerful vasodilator and abolished hypoxic pulmonary vasoconstriction; in intact ferrets it reduced pulmonary artery pressure (Ppa) substantially more than systemic pressure [2]. Given chronically, it also partially prevented the rise in pulmonary artery pressure, right ventricular hypertrophy and muscularization of pulmonary arterioles which develop when rats are exposed chronically to hypoxia; the pressure/flow relations of the lungs came close to those of normal lungs [3].

Our first aim in this study was to look in detail at the acute haemodynamic effects of ligustrazine in normoxic control (C) and chronically hypoxic (CH) rats; we measured the effect of increasing cumulative doses of the drug on pressure/flow (P/Q) relations. Previous evidence from isolated rings of pulmonary artery showed that ligustrazine acts in the absence of endothelium [4]. Our second aim was, therefore, to see if it was active in the whole isolated perfused pulmonary circulation in the absence of endothelial-derived relaxant factor (EDRF). We blocked synthesis of nitric oxide (NO), believed to be one EDRF [5], with an arginine analogue, L-nitro arginine methyl ester (L-NAME). This could be an important therapeutic point since the endothelium is frequently damaged in pulmonary vascular disease. The normal pulmonary vasculature is in a state of near complete dilatation. Thus to test a vasodilator substance, preconstriction is necessary; we used hypoxic vasoconstriction.

In the chronically hypoxic rat we have a model which in some ways resembles human pulmonary hypertension; Ppa is raised, the right ventricle is hypertrophied and new smooth muscle extends into the previously
non-muscularized or only partially muscularized alveolar arterioles (50 μm or less in diameter) [6, 7]. These features are also found in human chronic bronchitis [8]. However, the rat model lacks the endothelial damage and proliferation seen in hypoxic cor pulmonale [9, 10], while in more severe pulmonary vascular disease such as "primary" pulmonary hypertension even more severe damage is found.

Materials and methods

Young, 33 days old, male Wistar rats (specific pathogen free, Tuck's laboratories) were exposed for 3 weeks to 10% O₂ in a normobaric hypoxic chamber [11]. After pentobarbitone anaesthesia (60 mg·kg⁻¹, i.p.) the isolated perfused lungs of these CH rats (n=6) were perfused by a modification of HAnde's [12] method [13, 14] and compared with those of littermate control rats (n=5) kept in the same room in air. Lungs were ventilated with air+5% CO₂ (normoxia) or 2-3% O₂+5% CO₂, balance N₂ (hypoxia); end expiratory pressure was 2-3 mmHg. The lungs were perfused with 8-10 ml blood of normal pH (7.35-7.45, adjusted with sodium bicarbonate) at 38°C; blood for CH rats was taken from a donor since polycythaemia, present in CH rats, affects pulmonary vascular resistance (PVR) [15]. Normoxic and hypoxic ventilation was applied at 15-20 min intervals depending on the time taken to reach stable hypoxic pulmonary vasoconstriction (HPV) and a stable normoxic base line between hypoxic tests. As previously shown, this base line tends to rise slightly with time in C rats but much more so in CH rats for an unknown reason [13].

Pressure/flow line measurement

Blood flow was kept at 20 ml·min⁻¹ except when measuring pressure/flow (P/Q) lines. Left atrial or outflow pressure was kept at zero so that the alveolar pressure caused by positive pressure ventilation was the effective downstream pressure, or critical closing pressure, unless tone in small collapsible vessels took over this role [16-17], as it does during hypoxic vasoconstriction [18]; thus the lung was in West's zone 2. At constant flow, changes in mean Ppa represent changes in vascular resistance or in the effective downstream pressure; the nature of the changes was determined from the slope and position of the P/Q lines and is discussed in the text. Hypoxia caused a steep rise in pressure to a plateau which was usually sustained but occasionally slowly decayed. During this plateau doses of ligustrazine (20 mg·ml⁻¹, Fourth Pharmaceutical Laboratory factory, Beijing, China) were given; each caused a fall in Ppa to a new level. Figure 1 is a diagram which shows the protocol and typical responses. Sequential doses (0.5, 0.5, 1 and 2 mg given into the blood reservoir) were considered cumulative, as we had previously shown that the action lasted about 20 min; thus the final cumulative dose was 4 mg. Doses were given in 0.1-0.2 ml volumes which were too small to dilute the perfusate; similar volumes of 0.9% NaCl were given as controls. Two hypoxic tests were given first, as HPV tends to increase in the first few challenges, and ligustrazine was given during the third hypoxic challenge. P/Q lines were measured by plotting flow (Spectromed electromagnetic flow meter) against mean pressure (Druck Ltd transducers and Lectramed amplifier) on an XY recorder (Bryans model 2600 A4) at the points illustrated in figure 1; blood flow was reduced by alteration of the pump speed from 20 to 15, 10, 5 and 0 ml·min⁻¹. Lines were linear except at <5 ml·min⁻¹ and were extrapolated to the pressure axis to give an intercept.

Fig. 1. - A diagram of the experimental protocol. Ventilation was changed from air to 2% O₂ which caused a rise in pressure to a stable plateau. Ligustrazine was given into the reservoir where indicated in increasing cumulative doses; actual doses are marked (1). Pressure flow relations were taken at the hypoxic plateau and at each new lower level attained after the individual doses of ligustrazine. Ppa: pulmonary artery pressure.

Blockade of EDRF

In 7 normal rats (male Wistar, local stock) and a further 3 C and 4 CH littermates (as for the P/Q tests), isolated perfused lungs were set up and L-NAME (100 μg in 0.1 ml, final concentration ca. 10⁻⁴ M) was given into the reservoir when a consistent HPV had been established. After a 15-20 min wait to see whether L-NAME affected the normoxic pressure, ligustrazine was given into the reservoir during a further hypoxic test at peak HPV.

Statistics

Means and standard errors of the means (sEM) are quoted in text and figures. Differences between means were tested by paired or unpaired Student's t-test as appropriate; p values <0.05 were considered significant. Regression lines were calculated by the method of least squares.
**Results**

Although body weight of chronically hypoxic rats was less than that of controls (226±9 g cf 302±35, p<0.001), the same flow rate was used in both groups. We have previously shown that lung size and vascular volume are not dissimilar despite body growth retardation [6, 13]. Initial Ppa in 5 C rats was 18.1±1.2 mmHg (SEM) compared with 28.4±4.3 in 6 CH rats (p<0.05). After 1 h, when P/Q lines were measured, the values were 17.2±1.9 mmHg cf 37.5±3.3 (p<0.01) owing to the rise in normoxic Ppa of CH rats already mentioned. The rise in CH rats was significant (p<0.05). During the 2nd hypoxic response, volumes of 0.9% NaCl equivalent to those used for ligustrazine caused small depressions of the hypoxic plateau and ligustrazine reversed this in a dose dependent manner. In the 3rd hypoxic test Ppa rose to 35.1±8.5 in C rats and 56.7±12.9 in CH rats and ligustrazine reversed this in a dose dependent manner. Figure 2 shows the linear log-dose relationship in both groups (r=0.99). Baseline normoxic Ppa values were achieved after 4 mg cumulative dose in C rats and 2 mg in CH rats. In CH rats 2 mg or more brought the Ppa below the previous baseline normoxic value. On return to air ventilation there was no further fall in Ppa and no subsequent HPV could be demonstrated for 20 min after administration of ligustrazine.

Figure 3 shows mean P/Q lines for all rats, C on the left and CH on the right. Acute hypoxia caused an increase in slope and intercept in both groups but the intercept changes were greater in CH rats. Ligustrazine reduced slopes and intercepts in a dose dependent manner until the lines were similar to (C rats) or below (CH rats) those measured in normoxia. Figure 4 shows log-dose plots of the changes in slope and intercept (linear regression was 0.99 for all lines). It is apparent that, in CH rats, changes in intercept (shown above), which represent effective downstream pressure are significantly greater than in C rats (p<0.05) while changes in slope (shown below), which are a measure of vascular resistance, are similar.

L-NAME either caused no rise or only an occasional trivial rise in normoxic Ppa in the 7 C rats tested, as previously shown [19]; however, in the former study it systemically caused a rise in CH rats. A subsequent hypoxic challenge in the present C rats showed a greatly enhanced response as it did in both C and CH rats in the earlier study. Figure 5 shows that during this test, 4 mg ligustrazine greatly reduced Ppa as before but not to baseline pressure. Subsequent hypoxic tests gave reduced rises in Ppa but could be obtained within 5–10 min, unlike those in the above tests without L-NAME. In the further 3 C and 4 CH littermate rats L-NAME was given as just described but during the subsequent hypoxic test a series of doses (0.5, 0.5, 1, 2, total cumulative dose 4 mg) gave results similar to those seen in the P/Q experiments. The numbers were too few to compare C with CH rats but in 2 CH rats (and no C rat) the total dose brought the Ppa below the previous normoxic level during continued hypoxia.
and 3rd tracings yet another reduced hypoxic vasoconstriction is 

There is no time interval between the 2nd next hypoxic test 

The top line shows the rise in pulmonary 

pressure, 

NAME) 

Fig.

L-NAME methyl ester 

Ligustrazine caused a dose-dependent reduction in 

hypoxic pulmonary vasoconstriction in both control and 

chronically hypoxic rats; it appeared more effective in the 

latter. Moreover, it reduced the baseline normoxic 

Ppa in chronically hypoxic rats such that the increase 

in this pressure with time was abolished; thus, this rise, 

which can be extreme, is not caused by oedema but by 

vasoconstriction. It is not likely to be due to release of 

potassium ions through greater haemolysis in CH rats 

since we used normal blood for the perfusate in both 

groups. 

The greater decrease in intercept after ligustrazine in 

CH compared with C rats is of interest. These rats 

have newly developed muscle in very small arterioles 

(50 μm or less in diameter) which normally have little 

or no muscle. We have shown elsewhere that the main 
site of hypoxic vasoconstriction may move distally to 

these vessels in chronic hypoxia [18]. These small 

vessels may constrict and act as Starling resistors during 

hypoxia to form the effective downstream pressure or 
critical closing pressure; a lesser or similar effect of 
hypoxia on resistance may perhaps be attributed to 
distension of larger vessels due to the rise in Ppa 

caused by the intensely constricted small vessels. The 

present work suggests that ligustrazine acts on these 

small vessels, an important point for possible therapy 

because vasodilators must affect small intrapulmonary 

vessels as well as larger vessels. Differential effects 

between large and small vessels are well documented 

and it was recently shown that ligustrazine had a similar 
or possibly greater action on small compared with 

large intrapulmonary vessels [4, 20]. 

Ligustrazine did not reduce Ppa in CH rats to 
normoxic levels seen in C rats. It is evident that part 
of the raised Ppa in these rats, as in human pulmonary 
hypertension, cannot be resolved by reduction of tone 
because it is due to structural remodelling [7]; the small 
vessels are of normal external diameter but the lumen 
is narrowed by the new muscle bounded internally by 
a new elastic lamina [21]. Many attempts have been 
made to prevent these changes caused in animals by 
hypoxic exposure with vasodilator substances [22]. 

Several drugs, including ligustrazine [3], have attenuated 
the changes when given simultaneously with hypoxic 
exposure but only calcium channel inhibitors have 
brought about some attenuation after the changes have 
developed [23]. 

Our results in ferrets suggested that 

ligustrazine is a more potent dilator of pulmonary than 

systemic vessels [2]; this is an important point which 

needs substantiation in other circumstances. 

Many pulmonary vasodilator substances have failed clinically 

because they caused systemic hypotension. 

Our findings with L-NAME and the previous findings 
of Liu et al. [4] that ligustrazine continues to dilate 

preconstricted lung vessels after removal of endothelium, 

indicate that it is a non-endothelial-dependent 

vasodilator. This is an important therapeutic considera-

tion, because both pharmacological and pathological 
evidence suggest that the endothelium is often
severely damaged in human pulmonary hypertension [9, 10]. This is especially true in severe progressive cases, such as "primary pulmonary hypertension" where cardiac output is impaired and a reduction in pressure is imperative. Ligustrazine should be further investigated because it is effective orally, has a prolonged action and clinical tests in China have already been undertaken.

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References

2. Cai YN, Bee D, Barer GR. - Pulmonary vasodilator action of ligustrazine, active principle of a traditional Chinese remedy, in rats and ferrets. _Proceedings Chinese Academy Medical Sciences and Peking Union Medical College_, 1989, 4, 147-152.


Les effets de la ligustrazine, principe chimique d'une herbe médicinale chinoise ancienne, sur les relations pression/débit dans les poumons de rats isolés et perfusés. A. Oddoy, D. Bee, C. Emery, G. Barer. RÉSUMÉ: La ligustrazine, principe synthétisé d'une herbe médicinale chinoise dont l'effet vasodilatateur pulmonaire avait été démontré précédemment, a été testée chez des rats chroniquement hypoxiques et normoxiques. Les relations pression/débit et P/QO ont été mesurées dans les poumons isolés et perfusés au cours de la normoxie, de l'hypoxie et après correction de la vasoconstriction hypoxique par des doses croissantes de ligustrazine. Les lignes P/QO s'avèrent linéaires sur une large zone, et l'extrapolation sur l'axe des pressions donne un intercept qui est la pression effective en aval pour le débit. Chez les rats chroniquement hypoxiques, la forme de la ligne est plus verticale et l'intercept plus grand que chez les rats contrôle, ce que nous avons attribué à des artéiololes nouvellement muscularisées et toniques. L'hypoxie a provoqué une augmentation de la pente et de l'intercept dans les deux groupes, mais l'augmentation d'intercept est plus marquée chez les rats chroniquement hypoxiques. Dans les deux groupes de rats, des doses croissantes de ligustrazine, données pendant l'hypoxie continue, provoquent un éventail de lignes qui se déplacent progressivement vers la ligne normoxique contrôle. Chez les rats chroniquement hypoxiques, il suffisait de 2 mg de ligustrazine pour ramener la ligne à la position normoxique, alors que chez les contrôles il en fallait 4. Chez les rats chroniquement hypoxiques, la modification d'intercept avec chaque dose est plus importante que chez les rats contrôle. Ceci suggère que la ligustrazine provoque principalement une relaxation des muscles des petits vaisseaux collatéraux. L'action de la ligustrazine persiste à la fois chez les rats contrôle et chroniquement hypoxiques après administration d'un analogue de l'arginine, qui bloque la synthèse de l'oxyde nitrique, facteur relaxant endothélial. Ceci, ainsi que des observations antérieures, suggère que la ligustrazine est un vasodilatateur pulmonaire non dépendant de l'endothélium. _Eur Respir J_, 1991, 4, 1223-1227.