Effect of fenspiride on pulmonary function in the rat and guinea pig

D. BEE, E. A. LAUDE, C. J. EMERY and P. HOWARD
Department of Medicine, University of Sheffield, U.K.

(Received 9 June/24 October 1994; accepted 11 November 1994)

1. Fenspiride is an anti-inflammatory agent that may have a role in reversible obstructive airways disease. Small, but significant, improvements have been seen in airways function and arterial oxygen tension in patients with mild chronic obstructive pulmonary disease. These changes have been attributed to the anti-inflammatory properties of the drug. However, airways function can be improved by other means, e.g. improved ventilation/perfusion ratio or reduced airways resistance. The possibility that fenspiride may have actions other than anti-inflammatory was investigated in two animal species.
2. In the rat, actions on the pulmonary circulation were investigated in the isolated perfused lung, but fenspiride proved to be a poor pulmonary vasodilator, showing only a small reversal of the raised pulmonary artery pressure induced by hypoxia.
3. Ventilation was measured in the anaesthetized rat using whole-body plethysmography. Fenspiride caused no increase in ventilation or changes in arterial blood gases. However, a profound hypotensive action was observed with high doses.
4. The possibility that a decrease in airways resistance (R_{aw}) might occur with fenspiride was investigated in anaesthetized guinea pigs. Capsaicin (30 μmol/l) was used to increase baseline R_{aw} through bronchoconstriction. Fenspiride gave a dose-dependent partial reversal of the raised R_{aw}, and its administration by aerosol proved as efficacious as the intravenous route. In addition, the hypotensive side-effect found with intravenous injection was alleviated by aerosolized fenspiride.
5. An anti-bronchoconstrictor action of fenspiride could be one of the mechanisms involved in improving airways function and PaO_2, seen in mild chronic obstructive pulmonary disease.

INTRODUCTION

Fenspiride (Pneumorel, Biopharma, France) is a derivative of azaspirodecane [phenyl 8-oxa-diazi 3-8 spiro (4,5) decanone-2.HCl] with an M_r of 297 (Fig. 1). Fenspiride has been shown to be a non-steroidal, anti-inflammatory agent with anti-allergic and anti-bronchoconstrictor properties [1]. No β-agonist properties have been shown, although α_1-antagonist activity is present. In addition, as found with xanthines, fenspiride inhibits phosphodiesterase and increases cyclic AMP [1]. These properties indicate a possible important role in reversible obstructive airways disease. In a double-blind placebo-controlled study in patients with mild chronic obstructive pulmonary disease (COPD), there was an improvement in FEV_1 (forced expiratory volume in 1 s) and arterial oxygen tension (PaO_2) after 6 months' treatment with fenspiride; there was also a significant improvement in cough and sputum production [2].

These results, in COPD patients, were thought to be a result of the anti-inflammatory properties of the drug. *In vitro* fenspiride has moderate activity on histamine receptor binding, the production of free oxygen radicals and cyclo-oxygenase metabolism, all of which feature strongly in the lung's inflammatory responses [3–5]. *In vivo* fenspiride has proved effective against induced inflammation in small laboratory animals (for example, in SO_2-induced chronic bronchitis in rats and allergic rhinitis in guinea pigs [6]) as well as in human inflammatory conditions [7–9]. However, it is possible that gas exchange could have been improved by actions on either the pulmonary circulation or ventilation [through ventilatory control or reduced airways resistance (R_{aw})]. Indeed anti-bronchoconstrictor activity has been noted *in vitro*, when fenspiride reversed histamine constriction in guinea pig tracheal rings [4], and *in vivo*, when histamine- and serotonin- but not acetylcholine-induced constriction was suppressed by fenspiride [10].

In this study, acute responses of the respiratory apparatus to fenspiride have been examined. The effects on pulmonary circulation and ventilation were measured in the rat and the effects on airway resistance were measured in the guinea pig. The rat was used in pulmonary circulation and ventilation experiments because it has well-documented acute and chronic responses to hypoxia, hypoxia being a prominent complication of COPD. However, when

Key words: airways resistance, capsaicin, pulmonary artery pressure, ventilation.
Abbreviations: ANOVA, analysis of variance; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; HPV, hypoxic pulmonary vasoconstriction; NK, neurokinin.
Correspondence: Dr D. Bee, Department of Experimental Medicine, University of Sheffield, Beech Hill Road, Sheffield S10 2RX, U.K.

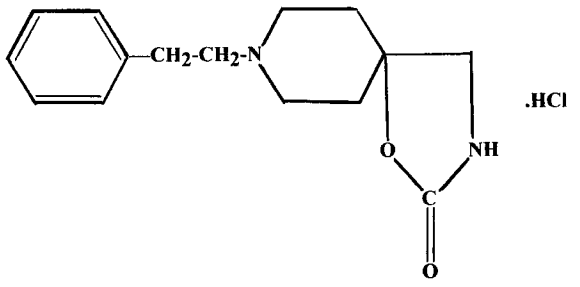

Fig. 1. Chemical structure of fenspiride hydrochloride. $M_r = 297$; pK = 8.

studying bronchoconstriction, the guinea pig is the most commonly used small laboratory animal as it has very reactive airways.

To determine fenspiride's vasoactive properties, we used the isolated perfused rat lung. As the pulmonary circulation is normally of low pressure with very little tone, it is necessary to raise pulmonary artery pressure (P_{pa}) in order to show any dilator properties of substances under investigation. This was achieved in two ways: first, acutely by ventilating lungs with low-oxygen mixtures and thus inducing hypoxic pulmonary vasoconstriction and, secondly, by inducing pulmonary hypertension by chronically exposing rats to a low-oxygen environment. Ventilation was assessed in the anaesthetized rat by whole-body plethysmography. Rats were anaesthetized with thiopentone (Inactin BYK), a long-lasting barbiturate anaesthetic that does not compromise blood pressure. Guinea pigs are known to have a highly reactive airway, so a model of capsaicin-induced increased R_{aw} was established in the guinea pig to investigate a possible bronchodilator action of fenspiride.

METHODS

Induction of chronic hypoxia

Male Wistar rats were obtained at 28 days old. After 5 days' acclimatization to laboratory conditions, they were divided into a control group and a second group which was subjected to a low-O_2 environment: 10% O_2 in a normobaric chamber for 2–3 weeks. Chronically hypoxic rats were used in the following procedures and compared with littermate normoxic controls.

Pulmonary circulation

The pulmonary circulation was investigated using the isolated perfused lung technique [11]. Twelve rats (body weight 200–250 g) were anaesthetized with Sagatal (sodium pentobarbitone, 60 mg/kg intraperitoneally). The trachea was cannulated and the animal exsanguinated via the inferior vena cava through a laparotomy. The rat was ventilated immediately and the anterior chest wall was removed, exposing the heart and lungs. The pulmonary artery and left atrium were cannulated and the lungs perfused at a constant flow rate of 20 ml/min with heparinized blood of normal haematocrit and temperature (37°C). Pulmonary artery pressure (P_{pa}) was recorded on a Bryans (U.K.) pen recorder using Viggo-Spectramed (Ohmeda, U.K.) pressure transducers (0–300 mmHg) with Lectromed (U.K.) amplification. Transducers were calibrated by a stepwise application of known pressures (10-mmHg steps, 0–70 mmHg) using a sphygmomanometer. Bolus fenspiride doses (0.1, 1, 10, 100, 200 and 400 μg and 1 and 2 mg) were given into the perfusion circuit close to the pulmonary artery.

Ventilation

Rats [normoxic, body weight 306 g (SEM 13), $n = 5$; chronically hypoxic, 260 g (SEM 8), $n = 6$] were anaesthetized with thiopentone (Inactin, BYK) and placed in a whole-body plethysmograph. The trachea and a femoral artery and vein were cannulated and connected to the outside of the plethysmograph. Blood pressure was measured from the femoral artery cannula [Viggo-Spectramed pressure transducers (0–300 mmHg)] with Lectromed amplification), which was also used to take arterial blood samples. Fenspiride infusions (total doses 1, 5, 10, 25, 50, 100 and 250 μg) were given via the femoral vein cannula. Minute ventilation (V_ε), arterial blood gases (PaO_2, $PaCO_2$) and pH were measured (Corning 158 blood gas analyser, U.K.) while breathing either air or 12% O_2.

Airway resistance

Dunkin–Hartley guinea pigs (body weight 450–550 g; $n = 37$) were anaesthetized with intraperitoneal urethane (1.5 g/kg). Blood pressure was monitored via a carotid artery cannula and intravenous infusions and bolus intravenous injections were introduced via a jugular cannula. Pleural pressure was measured via a cannula inserted into the pleural cavity between the fifth and sixth ribs [Viggo-Spectramed pressure transducers (0–300 mmHg) with Lectromed amplification] and airflow was measured from a tracheal cannula connected to a pneumotachograph. Flow was calibrated by passing air through a rotameter and plotting stepwise (0.5 l) increases in flow on the X–Y pen recorder. The animals were allowed to breathe spontaneously.

Airways resistance was measured from the pleural pressure–tracheal airflow relationship. A voltage proportional to lung volume was subtracted electronically from the pleural pressure and the resulting signal fed into one axis of an X–Y pen recorder while the airflow signal was fed into the other axis; the signals were in phase. Pressure flow lines were obtained, the slopes of which gave total airways resistance (R_{aw}).

Aerosols produced by a DeVilbis P1000 ultrasonic nebulizer were administered from an 800-ml clear plastic flask (with small openings at both ends) from which the animals breathed spontaneously via the tracheal cannula. Capsaicin (30 μmol/l) was given for 30 s, and 5 min later, when a sustained increase in R_{aw} was attained, aerosols of fenspiride (0.01, 0.1, 1 and 10 mg/ml for 1 min) or bolus intravenous injections (30, 100, 300 and 500 μg) were administered. Saline either intravenously or as an aerosol had little or no effect on R_{aw} after capsaicin. Usually R_{aw} increased slightly (see Fig. 3).

Blood pressure. Blood pressure was measured from a carotid artery catheter using a Viggo-Spectromed pressure transducer (0–300 cmH_2O) with Lectromed amplification. Results were continuously displayed on a Siemens (U.K.) SE 2600 UV recorder.

Statistics

Results are given as means ($\pm$SEM). Analysis of variance (ANOVA) or Student's paired or unpaired t-tests were used where appropriate. $P<0.05$ indicated significantly different values.

RESULTS

Pulmonary circulation

The mean normoxic P_{pa} of 16.0 mmHg (SEM, 1.1, $n=6$) in control rats was significantly less than that found in chronically hypoxic littermates (24.5 mmHg SEM 2.1, $n=6$; $P<0.01$). Ventilation with 2% O_2, 5% CO_2 in N_2 caused P_{pa} to rise to a new stable level (hypoxic pulmonary vasoconstriction, HPV); this increase was not significantly different between the two groups [+11.9 mmHg (SEM 1.9) for controls and +15.3 mmHg (SEM 1.5) for chronically hypoxic rats]. During normoxia fenspiride had a negligible effect on P_{pa} in both groups. During acute hypoxia lower doses (100–400 μg) caused an occasional small fall in P_{pa} in both groups, but the means were not significantly different from the saline control. Not until very large doses (1 and 2 mg) were used were significant reductions in P_{pa} found. In normoxic rats HPV was reversed by 2.80 (SEM 0.84) and 4.05 (SEM 0.83) mmHg [14.4% (SEM 4.3) and 24.5% (SEM 4.0)] after 1 and 2 mg of fenspiride respectively, while chronically hypoxic rats showed a greater reduction of 5.83 (SEM 1.03) and 4.55 (SEM 0.57) mmHg [31.5% (SEM 6.0) and 34.6% (SEM 8.2)].

Ventilation

No significant change occurred in ventilation or blood gases in normoxic and chronically hypoxic rats when fenspiride was given as an intravenous infusion (Table 1). No significant difference was found when both groups of animals were allowed to breathe reduced oxygen (12%). However, these experiments highlighted an adverse side-effect of the higher doses of intravenous fenspiride (Fig. 2). Blood pressure fell with increasing doses of fenspiride and was significant in chronically hypoxic rats (ANOVA, $P<0.001$). The final values after 250 μg of fenspiride were significantly different from the initial blood pressure in both groups ($P<0.02$ in controls and $P<0.003$ in chronically hypoxic rats, paired t-test). The hypotension developed more slowly but achieved a greater fall in normoxic than chronically hypoxic rats (34 versus 16 mmHg reduction respectively, not significant; Fig. 2).

Table 1. Ventilation data from normoxic and chronically hypoxic rats. The effect of intravenous fenspiride (F) during normoxic and acute hypoxic ventilation. V_E, minute ventilation. Values are means (SEM).

	Normoxia		Hypoxia		
	Control	250 μg F	Control	Hypoxia (FiO_2, 12%)	250 μg F
Control rats ($n=5$)					
V_E/100 g	64.6 (5.6)	72.7 (6.8)	77.5 (8.2)	91.3 (9.1)	87.3 (4.5)
PaO_2 (kPa)	13.3 (1.1)	15.4 (0.5)	15.2 (1.0)	11.2 (0.3)	10.9 (0.6)
Chronically hypoxic rats ($n=6$)					
V_E/100 g	62.3 (3.1)	56.4 (1.5)	56.9 (1.8)	62.6 (5.2)	67.4 (9.0)
PaO_2 (kPa)	11.4 (0.3)	12.0 (0.4)	11.6 (0.7)	8.7 (1.4)	9.3 (0.6)

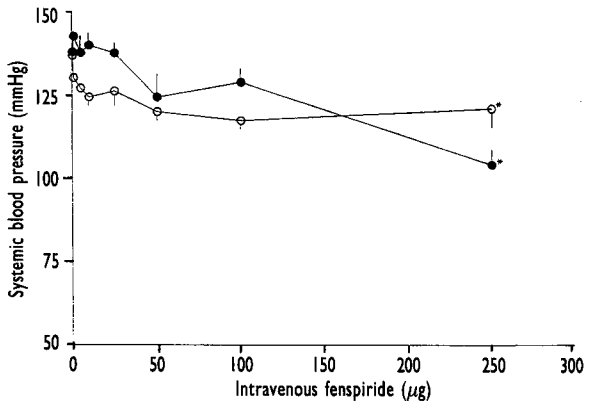

Fig. 2. Effect of intravenous infusions of fenspiride on the blood pressure of six normoxic (●) and six hypoxic (○) rats. The mean and standard error of the mean are plotted for each dose. *$P<0.05$, paired t-test.

Airways resistance

Fig. 3 shows an example of pressure–flow loops taken during an experiment with aerosolized fenspiride showing the effects of capsaicin, saline and 0.1 mg/ml fenspiride. Aerosolized capsaicin (30 μmol/l for 30 s) consistently produced an increase in airways resistance which was stable

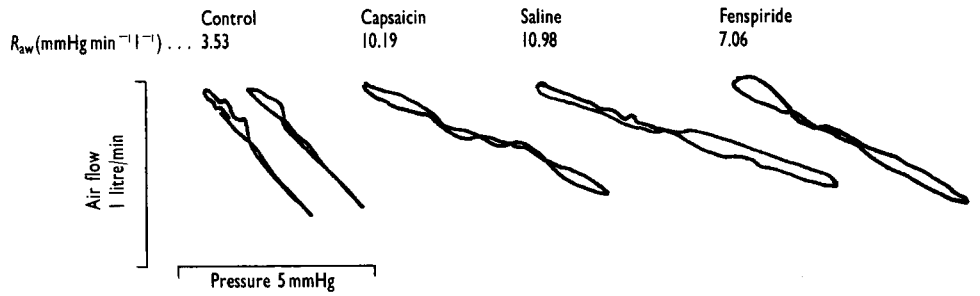

Fig. 3. Tracing of the pressure-flow loops taken from an experiment with aerosolized fenspiride in an anaesthetized guinea pig (body weight: 468 g). Measurements of airways resistance (R_{aw}) were made at 1-min intervals, but this figure shows the maximum effects of capsaicin ($t=5$ min), saline ($t=3$ min) and 0.1 mg/ml fenspiride ($t=5$ min). Fenspiride reversed the capsaicin-induced increase in R_{aw} by 52.6%. Absolute values of R_{aw} are given on the figure.

5 min after administration. The percentage increase varied with the sensitivity of the animal from 33.3% to 462.7%. Fig. 4 shows the absolute values for the effect of intravenous aerosolized fenspiride on raised R_{aw}. Aerosolized fenspiride 0.1, 1 and 10 mg/ml given for 1 min produced similar highly significant ($P<0.01$) percentage reductions in R_{aw} [76% (SEM 22), 58% (SEM 14) and 68% (SEM 9) respectively]. Aerosolized fenspiride at 0.01 mg/ml produced a smaller percentage reversal of R_{aw} [6% (SEM 3)]. Intravenous administration gave a clearer dose-related effect with percentage reversal increasing with increased dose [30, 100 and 300 μg gave 23% (SEM 7), 45% (SEM 7) and 90% (SEM 11) reversals respectively]. Both intravenous and aerosolized fenspiride had a similar time course of action, with the maximum effect being obtained after 5 min.

Blood pressure

Fenspiride given as bolus intravenous injections (in the above experiments) produced marked decreases in blood pressure (Table 2) which confirmed the observations found in the rat (Fig. 2). A small reduction [4.0 mmHg (SEM 1.3) and 3.6 mmHg (SEM 0.3); 9 and 7%] in blood pressure was also found at the higher aerosolized concentrations (1 and 10 mg/ml). A comparison of the blood pressures from those experiments giving a maximal reversal of airways resistance showed that the aerosol of fenspiride caused significantly ($P<0.01$) less hypotension than an intravenous injection (Table 2).

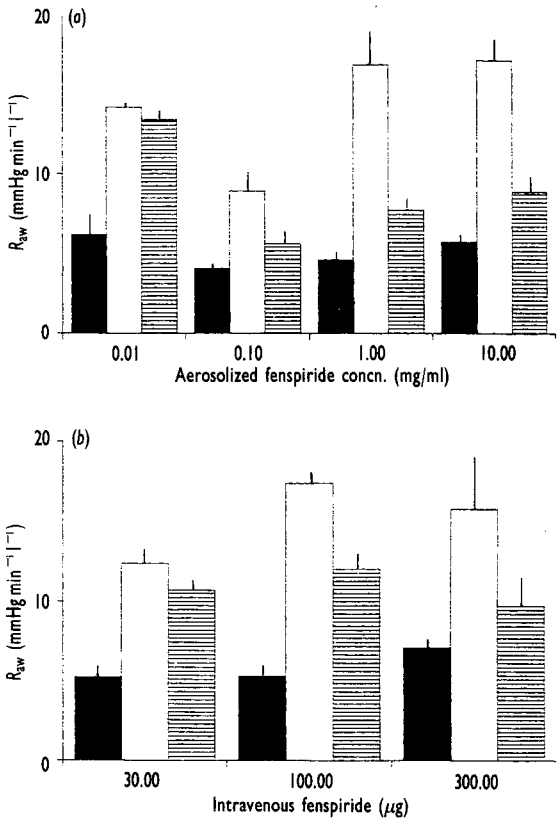

Fig. 4. Absolute airways resistance (R_{aw}) values (means ± SEM) are plotted showing the effects of aerosolized (*a*) and intravenous (*b*) fenspiride on capsaicin-induced bronchoconstriction in guinea pigs. ■, Basal R_{aw}; □, R_{aw} raised by 30 μmol/l capsaicin; ▤, effect of fenspiride ($n=5$ for all groups except 0.1 mg/ml aerosol, where $n=7$).

DISCUSSION

Acute fenspiride administration was seen to have no effect on ventilation or blood gases in normoxic or chronically hypoxic rats. In the rat pulmonary circulation, at high doses, there was a small vasodilatation which was more pronounced in the chronically hypoxic animals. Any slight increase in total pulmonary blood flow should contribute to increased PaO_2. However, fenspiride seems to be more effective during hypoxia, and preferential dilation of vessels in hypoxic units could cause a disruption of the ventilation/perfusion ratio [12, 13]. Thus fenspiride, along with other pulmonary vasodilators, is unlikely to account for improvements in blood gases in patients with heterogeneous lung disease, at least in the short term. It also must be

Table 2. Comparison of intravenous and aerosol routes of delivery on the hypotensive action of fenspiride. *Except for the 30-μg dose all intravenous responses were significantly greater than those with aerosol ($P<0.01$).

Aerosol route			Intravenous route		
n	Dose	Fall (%)	*n*	Dose	Fall (%)
5	1.0 mg/ml	8.68±1.1	5	30 μg	13.0±3.8
6	10.0 mg/ml	7.17±2.0	5	100 μg	17.5±0.7*
			5	300 μg	21.0±3.3*
			5	500 μg	23.6±2.0*

noted that the fenspiride levels inducing changes in animal P_{pa} are much higher than the therapeutic doses currently prescribed. Fenspiride, given either intravenously or as an aerosol, has proved to be an effective anti-bronchoconstrictor against capsaicin-induced increase in R_{aw}, although the increased R_{aw} is not completely reversed. The most likely mechanism for an improvement in arterial oxygenation would seem to be through the anti-bronchoconstrictor activity.

Capsaicin causes bronchoconstriction in both humans [14] and guinea pigs [15]. In the latter we have shown that its action is prolonged and that the bronchoconstriction achieves a stable phase after approximately 5 min. However, in man the bronchoconstriction is short-lived, being reversed in approximately 1 min. Capsaicin has several actions on the respiratory apparatus that may contribute to bronchoconstriction. Capsaicin stimulation of C-fibres [16] induces the release of neurokinins which cause both bronchoconstriction via contraction of airway smooth muscle [17] and microvascular leakage [18], which contributes to increased R_{aw} via airways obstruction. In addition, Fuller et al. [14] have shown a cholinergic component of capsaicin-induced bronchoconstriction.

Fenspiride could inhibit capsaicin-induced constriction by an action on either the synthesis or release of neurokinins or by competition for the neurokinin receptor sites, NK_2, on smooth muscle. Preliminary data regarding substance P bronchoconstriction do not support NK_2 receptor antagonism [19]. Consequently, it seems most likely that fenspiride could act to prevent synthesis or release of the neurokinins either directly or via a membrane-stabilizing effect. Antagonism of the NK_1 receptor could improve R_{aw} by reducing microvascular leakage. We are currently investigating the action of fenspiride against other bronchoactive agents, including specific NK_1 and NK_2 agonists. In addition, fenspiride is unlikely to be acting on cholinergic pathways, as it was unable to modify acetylcholine-induced constriction in guinea pig tracheal rings [10]. Maximal fenspiride effect caused approximately a 70% reversal of capsaicin bronchoconstriction. The residual increased R_{aw} may therefore be due either to the unaffected cholinergic component or to a fixed airways obstruction resulting from microvascular leakage. In summary, it is probable that fenspiride does not affect the entire spectrum of capsaicin's actions as only partial alleviation of capsaicin-induced bronchoconstriction has been found.

Our earlier studies on rats revealed that fenspiride causes a marked hypotension. The hypotension developed more slowly but was of greater magnitude in normoxic than in chronically hypoxic rats. This pattern of blood pressure response has been seen previously by the authors when testing rats with acute hypoxia, when it was usual to find that the control's blood pressure continued to decline after the chronically hypoxic rats had stabilized (D. Bee et al., unpublished work). This suggests either the detection of a fall in blood pressure by the carotid sinus is more sensitive in chronically hypoxic rats than in controls, allowing more efficient compensation for the effect, or that the vascular smooth muscle is less sensitive to a direct effect (e.g. fenspiride has a phosphodiesterase inhibitor action (1, 5]). However, the mechanism is, as yet, unknown. In the guinea pig the hypotension produced by intravenous treatment was markedly reduced when the drug was given via the aerosol route which still allowed full expression of the drug's bronchodilator effects. It could be argued that the hypotension, probably α_1 mediated [1], caused a sympathomimetic release of adrenaline which, by an action on the β_2-adrenergic receptors, could alleviate bronchoconstriction. It is unlikely that fenspiride acts in this manner as, when intravenous and aerosol doses were selected for their matched reductions in blood pressure, the former caused no significant change in R_{aw} while the latter induced a maximal anti-bronchoconstrictor effect.

In conclusion, we have shown that fenspiride is an effective anti-bronchoconstrictor agent against capsaicin-induced increased R_{aw} in the guinea pig. Other physiological mechanisms examined in the rat, such as the control of pulmonary blood flow and ventilation, which could increase arterial oxygen tension, are poorly affected by fenspiride. Should fenspiride act similarly in humans, the improvement in blood gases in COPD patients could be due to an effect on a reversible portion of the lung disease (i.e. an alleviation of bronchoconstriction) and consequently be of little benefit to those chronically hypoxic patients with fixed airways disease. The clinical importance of a hypotensive side-effect is crucial in the choice of administrative route. In this study the hypotension, most in evidence on intravenous administration, could be alleviated or abolished by administration of the drug via the aerosol route.

ACKNOWLEDGMENT

We thank Institut de Recherches Internationales Servier for generous financial support and supplies of fenspiride.

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