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Anti-platelet agents reduce morphological changes of chronic hypoxic pulmonary hypertension

Ingegerd M. Keith[1], James A. Will[2], Ryan J. Huxtable[3] and Kenneth Weir[4]

[1]School of Veterinary Medicine and [2]Department of Veterinary Science, University of Wisconsin, Madison, USA;
[3]Department of Pharmacology, University of Arizona Health Science Center, Tucson, USA;
[4]Department of Medicine, Minneapolis VA Medical Center, Minneapolis, USA

Summary. The pathophysiologic mechanism by which chronic hypoxia causes pulmonary hypertension is unknown. If anti-platelet agents, or other pharmacologic interventions, altered the pulmonary vascular changes induced by hypoxia, information concerning the pathogenesis of the pulmonary hypertension or the potential therapeutic usefulness of the drugs might be obtained. In Study 1, rats exposed to chronic hypobaric hypoxia (P_B = 520 mmHg) had a pulmonary arterial medial thickness of 6.7 ± 0.6 μ compared to 4.1 ± 0.2 μ * for control, normoxic rats (*$p<0.05$). Administration of dipyridamole (2mg/kg/day), or sulfinpyrazone (11 mg/kg/day) in the drinking water reduced the medial thickness to 5.0 ± 0.3 μ* and 5.4 ± 0.5 μ* respectively, thus suggesting the possible involvement of platelets in the response of the media to chronic hypoxia. In Study 2, hypoxic rats treated with the calcium blocker, flunarizine, were found to have less medial hypertrophy than a control group of hypoxic rats. This observation suggests that a decrease in transmembrane calcium flux may also reduce medial hypertrophy.

Key words: Hypoxia - Rats - Platelets - Pulmonary hypertension - Calcium blockers

Introduction

Exposure to chronic hypoxia is often used as a method to induce experimental pulmonary hypertension (Herget et al., 1978; Rabinovitch et al., 1979), and thus mimic the pulmonary hypertension which occurs in chronic hypoxic lung diseases in man. This condition is manifest physiologically by increased right ventricular and pulmonary artery blood pressures and morphologically by right ventricular hypertrophy and thickening of the media of small pulmonary arteries. The pathophysiologic mechanism by wich hypoxia causes pulmonary hypertension is not known. Despite earlier indications (Hauge, 1968; Hauge and Melmon, 1968), it does not appear that platelets help to mediate the acute pulmonary vascular pressor response to hypoxia in rats (McMurtry et al., 1978) or dogs (Weir et al., 1976). However, the number of circulating platelets is reduced by chronic hypobaric hypoxia in man (Gray et al., 1975), cattle (Genton et al., 1970), mice (Birks et al., 1975) and rats (DeGabriele and Pennington, 1967). Given that these platelets can be sequestered in the lungs (Gray et al., 1975), it is possible that they may contribute to the increase in pulmonary vascular resistance caused by chronic hypoxia. There is evidence that platelets are involved in the pulmonary hypertension induced by microemboli (Mlczoch et al., 1978) and by subclavian artery to pulmonary artery shunts (Van Benthuysen et al., 1981). If antiplatelet agents reduce the pulmonary vascular changes caused by chronic hypoxia, this might indicate a role for platelets in the pathogenesis of hypoxic pulmonary hypertension and the findings might be of potential therapeutic importance. Consequently pulmonary hypertension was induced in rats by exposure to chronic hypoxia. Some of these rats were treated with dipyridamole or sulfinpyrazone. Other rats exposed to chronic hypoxia were treated with the calcium channel blocker, flunarizine (Van Neuten and Janssen, 1973; Borgers et al., 1980) in an attempt to elucidate the mechanisms underlying morphologic changes in chronic hypoxic pulmonary hypertension.

Materials and methods

A total of 72 male Sprague Dawley rats were used in the experiment. Rats which were made hypoxic were housed for two weeks in a hypobaric chamber at 520 mm Hg, simulating an altitude of 3,105 meters, and were then killed while hypoxic. The chamber was returned to ambient air pressure for 30 minutes daily for feeding and

Offprint requests to: Ingegerd M. Keith, Ph.D., School of Veterinary Medicine, 2015 Linden Drive West, Madison, WI 53706, USA

cleaning. All drugs were dissolved in the drinking water and fresh solutions were made each day. Drug treatment started five days before the start of hypoxia and continued throughout the experiment. Water was restricted in all groups to the amount drunk by the treated rats and commercial rat food was given ad lib.

In the first study, 42 rats (150-200g) were divided into four groups: 1) normoxia without drug (n = 10), 2) hypoxia without drug (n = 10), 3) hypoxia with dipyridamole, 2 mg/kg/day, (n = 11) (Persantine, Boehringer Ingelheim), 4) hypoxia with sulfinpyrazone, 11 mg/kg/day, (n = 11) (Anturane, Ciba-Geigy). In the second study 30 rats (325-350g) were divided into three groups (n = 10 for each): 1) normoxia without drug, 2) hypoxia without drug, 3) hypoxia with flunarizine, 4mg/kg/day (Sibelium, Janssen Pharmaceutica). In all the studies the rats were killed by the intraperitoneal injection of sodium pentobarbital. The dorsal aorta was cut to remove blood from the heart and lungs. The lungs were instilled in the closed chest with Bouins fixative through a tracheal cannula at a pressure of 12-15 cm water (Wagenvoort and Wagenvoort, 1977). Sagittal paraffin sections were stained with hematoxylin-eosin for morphology and Millers elastic stain for morphometry (Miller, 1971). The hearts were removed, opened to evacuate trapped blood and fixed in 10% formalin. The right ventricle (RV) was separated from the left ventricle and interventricular septum (LV + S) (Fulton et al., 1952) and both components were weighed separately. Right ventricular hypertrophy was indicated by decreasing (LV + S)/RV weight ratios. To determine pulmonary arterial changes, the distance between the external and internal elastic laminae was measured at four equidistant sites in cross-sectioned small arteries, and the mean was calculated to determine the average medial thickness (Keith and Will, 1981). Ten arteries per animal were measured, ranging in outer diameter from 50-100 microns. Analysis of variance and Duncan's multiple range test were used to assess differences between groups. The tissues were coded so that all measurements were made without knowledge of the experimental group from which the tissue was derived.

Results

Hypoxic control rats had significantly increased medial thickness of the small pulmonary arteries and significantly smaller (LV + S)/RV ratios than had normoxic controls (Table 1). The decreased (LV + S)RV ratios were caused by an increase in the weight of the right ventricle alone. The hypoxia-induced medial thickening was not prevented, but was significantly reduced by dipyridamole, sulfinpyrazone and flunarizine (Table 1). None of these drugs had any effect on (LV + S)/RV ratios.

The hypoxic rats in the first study did not gain body weight as readily as the normoxic controls, whereas the older rats in the second study gained little weight and showed no difference in body weight between any groups (Table 1). There was no effect on body weight, as all hypoxic groups with or without drug had similar weights within both experiments.

Discussion

Chronic hypoxia produced an increase in medial

Talbe 1. The effects of dipyridamole, sulfinpyrazone, and flunarizine on hypoxia induced cardiopulmonary changes in rats

	Treatments	Number of rats	Body weight (grams)	Lung arterial medial thickness (microns)	Weight ratios Left ventricle plus septum to right ventricle
Study I					
	Normoxia only	10	303 ± 6	4.15 ± 0.23	3.83 ± 0.13
	Hypoxia only	10	270 ± 3	6.74 ±0.59*	3.04 ± 0.10*
	Hypoxia with dipyridamole	11	265 ± 5*	5.03 ± 0.29*†	2.94 ± 0.12*
	Hypoxia with sulfinpyrazone	11	265 ± 4*	5.42 ± 0.47*†	2.98 ± 0.14*
Study II					
	Normoxia only	10	375 ± 2	2.91 ± 0.15*	3.46 ± 0.12
	Hypoxia only	10	364 ± 6	6.23 ± 0.18	2.90 ± 0.15
	Hypoxia with flunarizine	10	372 ± 7	5.25 ± 0.34*†	2.73 ± 0.09*

* different from the normoxic control group; $p < .05$
† different from the hypoxic untreated group; $p < 0.05$

means and standard erros are shown

thickness of the pulmonary arteries and a decrease in the (LV + S)/RV ratio, as anticipated. The administration, of dipyridamole, sulfinpyrazone or flunarizine reduced the medial thickness of the arteries toward normoxic levels but the ratio (LV + S)/RV was unchanged. The reason for this dissociation between the medial thickness changes and the right ventricular hypertrophy is not clear. It may be there was enough hypoxic pulmonary vasoconstriction to cause pulmonary hypertension and stimulate right ventricular hypertrophy, but that the increase in arterial medial thickness requires some platelet-derived factor such as that described by Ross et al. (1974).

Dipyridamole is known to alter platelet function in vitro (Cucuiano et al., 1971) and in vivo (Harker and Slichter, 1970). However, it has also been demonstrated that dipyridamole is equally effective in the inhibition of acute hypoxic pulmonary vasoconstriction in the dog in the presence, or virtual absence, of circulating platelets (Weir et al., 1976). Dipyridamole may be causing direct vasodilatation through its ability to increase levels of both cyclic AMP and prostacyclin. Consequently, the reduction in medial thickness observed in the hypoxic rats treated with dipyridamole does not necessarily incriminate platelets as a factor in the stimulation of medial hypertrophy. The action of sulfinpyrazone is more likely to be directly related to its effect on platelet behavior, through the inhibition of thromboxane synthesis (Patrono et al., 1980). The similar results of both «anti-platelet» agents makes it possible that platelets are involved in the pulmonary vascular response to chronic hypoxia. This suggestion is strengthened by a study which demonstrated increased platelet activation in patients with chronic obstructive airways disease (Nenci et al., 1982). Platelet production of malondialdehyde was reduced, which occurs in «overstimulated» platelets, and plasma levels of ß - thromboglobulin, an indicator of platelet activation, were increased. After administration of dipyridamole for ten days these markers of platelet activation returned close to normal levels. Other experiments suggest that platelets have a role in the increased pulmonary vascular resistance occurring in subclavian artery to pulmonary artery shunts (Van Benthuysen et al., 1981).

Calcium channel blockers, such as verapamil, have been shown to reduce the acute pulmonary pressor response to hypoxia in the isolated perfused rat lung (McMurtry et al., 1976) and the anesthetized dog (Tucker et al., 1976; Archer et al., 1985). The administration of verapamil (4 mg i.p. twice daily) to rats exposed for 20 days to chronic hypoxia reduced the severity of right ventricular hypertrophy in another experiment (Davidson et al., 1978), but medial thickness was not examined. In the present study the calcium channel blocker, flunarizine, was found to reduce the degree of pulmonary arteriolar medial hypertrophy stimulated by hypoxia, but did not prevent the increase in right ventricular weight. The variation in the results could be secondary to the differences in the methods of drug administration, the dose used, the length of the studies, or the pharmacology of the two calcium blockers. It seems possible however, that the normal response of the pulmonary vasculature to chronic hypoxia, like that to acute hypoxia, requires transmembrane calcium flux. This could involve the reactivity of the smooth muscle itself, the release of a constrictor mediator, or the aggregation of platelets. Calcium channel blockers have been reported to inhibit platelet aggregation (Ono and Kimura, 1981), and in this respect flunarizine might mimic the actions of dipyridamole and sulfinpyrazone.

The results observed in these hypoxic rats suggest that platelets may be involved in the development of pulmonary arterial medial hypertrophy. The calcium channel blocker, flunarizine, produced a modest reduction in medial hypertrophy, in keeping with the effects of verapamil on the acute pulmonary pressor response to hypoxia (McMurtry et al., 1976; Tucker et al., 1976). These observations, together with the finding that nifedipine will inhibit experimentally induced bronchoconstriction in the guinea pig (Fanta et al., 1982), and exercise-induced asthma in man (Cerrini et al., 1981), support the use of calcium channel blockers in some patients with pulmonary hypertension secondary to chronic hypoxic lung disease (Kennedy et al., 1984; Muramoto et al., 1985).

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