Ventilation with the noble gas argon in an *in vivo* model of idiopathic pulmonary arterial hypertension in rats

Idiopathic pulmonary arterial hypertension (PAH) is a rare and progressive disease with high morbidity and mortality.[1] Endothelial dysfunction and obstructive vascular remodeling produce sustained high pressure in the pulmonary arterial system resulting in increased right ventricular (RV) afterload and hypertrophy. Maladaptive response to the elevated pulmonary vascular resistance (PVR) can promote RV failure and ultimately death. Although long-term survival in patients with PAH has significantly improved in the past two decades, the clinical burden of the disease is still high, with a 5-year survival rate of approximately 50%.[1] Therapies for PAH are mainly endothelin antagonists, for example bosentan (a non-selective endothelin-1 (ET-1) receptor antagonist) and ambrisentan (a selective ETA receptor antagonist). In general, vasodilators that improve hemodynamics and functional capacity ameliorating the quality of life have no definitive curative effects. Recently, the noble gas Argon (Ar) has been proposed as a pulmonary vasodilator agent.[2]

Suleiman et al.[2] for the first time described the vasodilating effects of 74% Ar in rat isolated perfused lungs and precision-cut lung slices from both rats and humans. In this *ex vivo* and *in vitro* study, the authors showed that treatment with Ar attenuates ET-1 induced vasoconstriction. Specifically, the effects of Ar can be summarized as:

a) Attenuation of the increase in capillary pressure and post-capillary resistance induced by ET-1 in isolated perfused lungs, although no reduction in pulmonary arterial pressure or PVR was detected;

b) Reduction of ET-1-induced contraction in both rat and human pulmonary arteries, measured as intra-vessel area extension in the precision-cut lung slices;

c) Reduction ET-1-induced lung edema in rat isolated perfused lungs.

In the present study we investigated whether prolonged inhalation of 70% Ar has pulmonary vasodilating effects in an *in vivo* rat model of severe PAH.

All procedures involving animals and their care fulfilled national and international laws and policies (approval No. 202/2017-PR; approval date 6/3/2017; Legislative Decree No. 76/2014-B, Italian Ministry of Health). PAH was induced in 11 male Wistar rats (6–8 weeks, Charles River, Calco, Italy) weighting 306 ± 6 g by subcutaneous injection of 60 mg/kg monocrotaline (Sigma-Aldrich, Inc., St. Louis, MO, USA), as previously described.[3]

Four weeks after monocrotaline injection, animals were randomized into: Ar, 24-hour inhalation of 70% Ar and 30% oxygen (O_2) ($n = 5$) or AIR, 24-hour inhalation of room air ($n = 4$). Ar mixture (SIAD, Bergamo, Italy) was delivered through a 200-L chamber prefilled with the gas (temperature 24–26°C, standard light-dark cycle). Total gas flow was measured with a flowmeter (1–10 L/min; Ohmeda, Selectatec, Sacem s.r.l, Cremona, Italy). Soda lime was added as a CO_2 scavenger.

After 24-hour treatment, echocardiography was performed (Arietta V70, Hitachi-Aloka, Milano, Italy) inside the chamber with the animals anesthetized. As previously described,[3] pulsed-wave Doppler of pulmonary outflow was recorded in parasternal short-axis view at the level of the great vessels and pulmonary artery acceleration time was measured as the time of systolic flow to peak pulmonary outflow velocity. The RV stroke volume (RVSV) and cardiac output (RVCO) were calculated by the formulas: $RVSV = \pi \times (\text{RV outflow tract}/2)^2 \times \text{RV outflow tract velocity time}$, $RVCO = SV \times \text{heart rate}$. RV catheterization (Millar SPR71, AD Instruments Ltd., Oxford, UK) was performed through the right jugular vein during 70% Ar inhalation delivered by a nose mask. RV systolic pressure was measured as surrogate of pulmonary artery systolic pressure. Before euthanasia, blood was collected (0.3 mL) for the measurement of plasma concentrations of N-terminal proatrial natriuretic peptide by a validated enzyme-linked immunosorbent assay kit (Biomedica BI-20892, Vienna, Austria). Rats were then euthanized, and lungs were excised, weighted and wet-to-dry ratio was calculated. Data are shown as mean ± standard error or median and [Q1–Q3]. Student's *t*-test or Mann-Whitney *U* test was used as appropriate.

Nine of eleven animals survived until the end of the experiment (29 days). Two rats died 26 days after monocrotaline injection, during the development of PAH. Echocardiographic variables showed a mild not significant improvement in Ar group compared to AIR group (pulmonary artery acceleration time, Ar: 17.7 ± 0.6 ms *vs*. AIR: 14.6 ± 2.3 ms, $P = 0.2$; RVCO, Ar: 69.6 ± 7.0 mL/min *vs*. AIR: 60.3 ± 18.9 mL/min, $P = 0.6$; RVSV, Ar: 0.19 ± 0.0180 mL *vs*. AIR: 0.15 ± 0.035 mL, $P = 0.3$). RV systolic pressure slightly decreased in Ar group compared to AIR group (Ar: 87.6 ± 10.1 mmHg *vs*. AIR: 101.8 ± 17.7 mmHg, $P = 0.5$). The median plasma concentration of N-terminal proatrial natriuretic peptide moderately decreased in Ar group compared to AIR group (Ar: 2.2 nM *vs*. AIR: 4.2 nM, $P = 0.7$). No differences between groups in the lungs wet-to-dry ratio were found (Ar: 5.0 ± 0.1 *vs*. AIR: 4.9 ± 0.1, $P = 0.8$).

To our knowledge, this is the first report studying *in vivo* the pulmonary hemodynamic effects of Ar inhalation in an animal model of PAH. No Ar-induced pulmonary vasodilating properties were shown, despite encouraging data from earlier investigations in *ex vivo* models.[2]

Besides the proven neuroprotective effect of Ar, its vasodilating effect in lung vessels is still debated. Earlier *in vivo* study showed no hemodynamic effect on both systemic and pulmonary circulation in healthy pigs subjected to 4 hour ventilation with 70% Ar in O_2.[4] Similarly, ventilation with Ar did not change significantly hemodynamics, including PVR, and respiratory variables before and after lung transplantation in pigs compared to control ventilation with N_2/O_2.[5] These findings are consistent with our previous observations in a swine model of cardiac arrest and cardiopulmonary resuscitation where ventilation with Ar did not affect pulmonary capillary wedge pressure, pulmonary arterial pressure, CO and thus the

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deriving PVR (Ar: 4.0 ± 0.4 *vs*. AIR: 3.1 ± 0.4, $P = 0.2$, after 4 hours of ventilation) compared to ventilation.[6]

Despite the current study proves only a mild and/or no vasodilating effects of Ar ventilation, no clear detrimental effect has been detected also in an established rat model of PAH. This important observation will eventually justify further investigation of this novel approach in terms of dose and timing of administration from the onset of the disease.

As the main limitation of the study, we acknowledged that little different O_2 concentration was used in Ar and AIR groups (30% and 21% respectively).

In conclusion, this *in vivo* study showed a lack of effects on pulmonary hemodynamics in PAH. The confirmed absence of detrimental effects of Ar treatment in this setting strongly supports other investigations in organ preservation and its potential clinical applicability in other pathological conditions.

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doi: *10.4103/2045-9912.314333*

How to cite this article: *De Giorgio D, Magliocca A, Fumagalli F, Novelli D, Olivari D, Staszewsky L, Latini R, Ristagno G. Ventilation with the noble gas argon in an in vivo model of idiopathic pulmonary arterial hypertension in rats. Med Gas Res 2021;11(3):124-125.*
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Date of submission: November 9, 2020
Date of decision: November 15, 2020
Date of acceptance: November 18, 2020
Date of web publication: April 27, 2021