Vitamin D deficiency, a potential cause for insufficient response to sildenafil in pulmonary arterial hypertension

Received: 27 April 2021
Accepted: 28 July 2021

To the Editor:

Phosphodiesterase 5 inhibitors (PDE5i), such as sildenafil and tadalafil, are frequently used to treat pulmonary arterial hypertension (PAH) [1]. Combined therapy of PDE5i with drugs acting *via* other signalling mechanisms, *i.e.* the endothelin pathway, is currently recommended as it provides better clinical outcomes than monotherapy with PDE5i [1, 2]. The soluble guanylyl cyclase (sGC) stimulator riociguat is an interesting alternative to PDE5i, with a different mode of action but on the same signalling pathway. Recently, the results of the REPLACE study show that, in patients remaining at intermediate risk of 1-year mortality after PDE5i treatment, switching to riociguat is beneficial in terms of clinical improvement and risk status as compared with PDE5i maintenance therapy [3].

There are theoretical reasons and empirical evidence to suggest that some patients with PAH may have a treatment response suboptimal to PDE5i but preserved to riociguat. Phosphodiesterase 5 degrades cyclic guanosine monophosphate (cGMP), which mediates the vasodilator and antiproliferative effects of nitric oxide (NO). Thus, PDE5i inhibits cGMP degradation and hence potentiates the effects of endogenous NO. Failure of PDE5i is presumed to be the consequence of low basal NO–sGC–cGMP activity, which is known to occur in PAH [4]. In contrast, riociguat increases intracellular cGMP levels by directly stimulating sGC, independent of available NO, but also sensitises sGC to NO. We have recently reported in an animal model that severe deficiency of vitamin D (vitD) further impaired endothelial-derived NO activity in PAH, and that calcitriol, the active form of vitD, can restore endothelial function [5]. Likewise, several reports have shown that vitD regulates NO activity in systemic arteries in both human and animal models [6].

Herein, we compared the responses to sildenafil and riociguat in isolated pulmonary arteries from rats with PAH that had been exposed to a vitD-free diet for 8 weeks with those after restoring vitD status for the last 3 weeks.

VitD deficiency led to a poor vasodilator response to sildenafil *ex vivo*, which can be reverted by restoring vitD levels (figure 1a). Remarkably, the response to riociguat is unaffected by the vitD status (figure 1b). Thus, these data indicate that vitD deficiency reduces the apparent basal NO-dependent cGMP production, decreasing the response to sildenafil in rats with PAH.

In Spanish and Japanese cohorts of PAH patients, 95% and 92% of them, respectively, showed vitD deficiency (serum 25(OH)vitD <20 $ng \cdot mL^{-1}$) and 70% and 42%, respectively, had severely decreased levels (<10 $ng \cdot mL^{-1}$) [7, 8]. 70% of the patients in the Spanish cohort also showed subsequent secondary hyperparathyroidism. Moreover, reduced bone mineral density and secondary hyperparathyroidism was also found in 80% and 55% of patients, respectively, in a Swiss PAH cohort [9]. We speculate that reduced vitD levels in PAH might partially account for the limited efficacy of sildenafil in some patients. Thus, we retrospectively compared the 25(OH)vitD levels in serum samples of PAH patients that responded *versus* those who did not respond to PDEi. Serum samples were obtained from the Spanish National PH Biobank and clinical data from the REHAP registry. Among the 113 PAH patients with available 25(OH)vitD levels, 67 had idiopathic, hereditary or drug-induced PAH [7], and 46 had associated PAH. 30 of them were on PDE5i as monotherapy and could be classified into responders or

Shareable abstract (@ERSpublications)
Vitamin D deficiency causes a reduced response to sildenafil but not to riociguat in rats with PAH. Lower levels of vitamin D were also retrospectively associated to insufficient response to PDE5 inhibitors in PAH patients. https://bit.ly/3rZDcTF

Cite this article as: Callejo M, Blanco I, Barberá JA, *et al*. Vitamin D deficiency, a potential cause for insufficient response to sildenafil in pulmonary arterial hypertension. *Eur Respir J* 2021; 58: 2101204 [DOI: 10.1183/13993003.01204-2021].

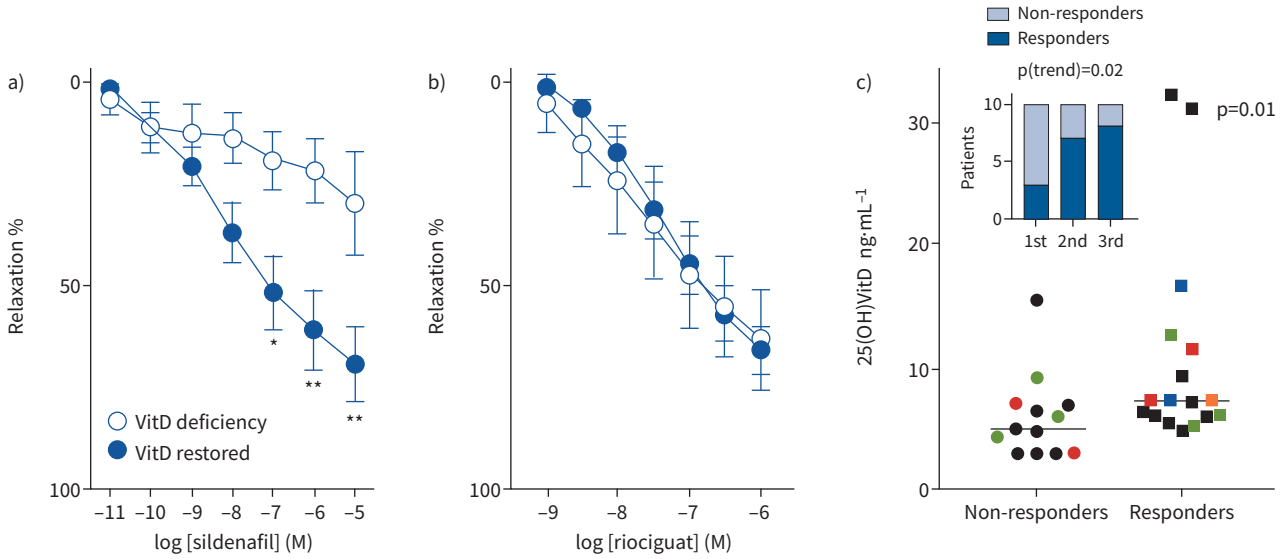

FIGURE 1 a, b) Wistar rats on vitamin D (vitD)-free diet for 5 weeks and further exposed to Su5416 (20 mg·kg^{-1}) and hypoxia (10% O_2) for an additional 3 weeks were returned to normoxia and randomised to either maintenance on vitD-free diet (vitD deficiency; n=8) or a single oral dose of 100 000 UI·kg^{-1} vitD_3 plus standard diet (vitD restored; n=9) for 3 weeks (final 25(OH)vitD serum levels were mean±SEM 4.3±0.3 and 70.2±7.3 ng·mL^{-1}, respectively) [10]. Pulmonary arteries from these animals were isolated, contracted with 5-HT (30 µmol·L^{-1}), and cumulative concentration–response curves to sildenafil (a) and riociguat (b) were performed in a wire myograph [5]. Results (presented as mean±SEM) indicate percent reversal of 5-HT-induced contraction. *: p<0.05; **: p<0.01 (t-test). c) Pulmonary arterial hypertension (PAH) patients treated with phosphodiesterase 5 inhibitor as monotherapy were classified into responders (n=17) and non-responders (n=13). 25(OH)vitD serum levels (scatter plot and median) were compared between the two groups with a Mann–Whitney test. PAH subtype is identified by colours: idiopathic (black), familial (red), portal hypertension (green), connective tissue disease (blue) and congenital heart disease (orange). The inset shows the number of non-responders and responders in the first, second and third tertile of 25(OH)vitD levels.

non-responders. Responders (n=17) were those meeting the three following criteria 12 months after PDE5i treatment initiation: 1) alive and free of lung transplant, 2) without clinical worsening (*i.e.* without treatment modification) and 3) improved risk score or remaining in low-risk profile. All others (n=13) were considered non-responders. Interestingly, responders to sildenafil had significantly higher 25(OH)vitD levels than non-responders (figure 1c). We also analysed the distribution of responders across tertiles of 25(OH)vitD levels. Similarly, there was a significant increase in the percentage of responders with increasing 25(OH)vitD (inset in figure 1c).

In conclusion, vitD deficiency causes a poor vasodilator response to PDE5i but preserved response to riociguat in rats with PAH. The high prevalence of vitD deficiency worldwide, which seems to be even higher in at least some patients with PAH, and the lower levels of 25(OH)vitD in non-responders to PDE5i compared to responders, suggest that this deficiency may cause insufficient response to PDE5i in some patients with PAH, a possibility that remains to be tested. Therefore, in addition to recovery of optimal vitD status being indicated to restore calcium homeostasis and prevent bone fractures in those PAH patients with severe deficiency, it might help to improve responsiveness to PDE5i.

María Callejo[1,2,3], Isabel Blanco[2,4], Joan Albert Barberá[2,4] and Francisco Perez-Vizcaino[1,2,3]

[1]Dept of Pharmacology and Toxicology, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, Spain. [4]Dept of Pulmonary Medicine, Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona, Spain.

Corresponding author: Francisco Perez-Vizcaino (fperez@med.ucm.es)

All individual participant data that underlie the results reported in this article will be available on request.

Conflict of interest: M. Callejo has no competing interests. I. Blanco has received honoraria from Actelion, Janssen and MSD. J.A. Barberá has received grants and honoraria from Actelion, Janssen, MSD and GSK. F. Perez-Vizcaino has received a lecture fee from Actelion.

Support statement: This study was supported by grants from Ministerio de Economía y Competitividad (SAF2016-77222-R and PID2019-107363RB-I00) and Fundación Contra la Hipertensión Pulmonar (Empathy), and an unrestricted grant from MSD. Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Galie N, Channick RN, Frantz RP, *et al.* Risk stratification and medical therapy of pulmonary arterial hypertension. *Eur Respir J* 2019; 53: 1801889.
2 Galie N, Barbera JA, Frost AE, *et al.* Initial use of ambrisentan plus tadalafil in pulmonary arterial hypertension. *N Engl J Med* 2015; 373: 834–844.
3 Hoeper MM, Al-Hiti H, Benza RL, *et al.* Switching to riociguat *versus* maintenance therapy with phosphodiesterase-5 inhibitors in patients with pulmonary arterial hypertension (REPLACE): a multicentre, open-label, randomised controlled trial. *Lancet Respir Med* 2021; 9: 573–584.
4 Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1995; 333: 214–221.
5 Callejo M, Mondejar-Parreno G, Morales-Cano D, *et al.* Vitamin D deficiency downregulates TASK-1 channels and induces pulmonary vascular dysfunction. *Am J Physiol Lung Cell Mol Physiol* 2020; 319: L627–L640.
6 Kim D-H, Meza CA, Clarke H, *et al.* Vitamin D and endothelial function. *Nutrients* 2020; 12: 575.
7 Callejo M, Mondejar-Parreno G, Esquivel-Ruiz S, *et al.* Total, bioavailable, and free vitamin D levels and their prognostic value in pulmonary arterial hypertension. *J Clin Med* 2020; 9: 448.
8 Tanaka H, Kataoka M, Isobe S, *et al.* Therapeutic impact of dietary vitamin D supplementation for preventing right ventricular remodeling and improving survival in pulmonary hypertension. *PLoS One* 2017; 12: e0180615.
9 Ulrich S, Hersberger M, Fischler M, *et al.* Bone mineral density and secondary hyperparathyroidism in pulmonary hypertension. *Open Respir Med J* 2009; 3: 53–60.
10 Callejo M, Morales-Cano D, Mondejar-Parreno G, *et al.* Restoration of vitamin D levels improves endothelial function and increases TASK-like K^+ currents in pulmonary arterial hypertension associated with vitamin D deficiency. *Biomolecules* 2021; 11: 795.