Phosphodiesterase - type 3 inhibitor cilostazole potentiates cAMP generation and antiproliferative effects of treprostinil in pulmonary arterial smooth muscle cells from patients with pulmonary hypertension

L. H. Clapp, N. Orie and Jigisha. Patel

INTRODUCTION

The prostacyclin class of drugs are used to treat pulmonary arterial hypertension, and while evidence suggests they improve survival, these agents eventually stop working. Thus ways are being sort to improve their clinical efficacy. We hypothesise that prostacyclin action could be enhanced by inhibition of phosphodiesterase type 3 (PDE3), a major regulator of cyclic AMP levels in the lung, whose activity appears increased in pulmonary hypertension.[1]

EXPERIMENTAL APPROACHES

Distal pulmonary arterial smooth muscle cells (PASMCs) isolated from the lungs of patients (n=6) suffering from idiopathic pulmonary arterial hypertension (IPAH) and rat tail artery were used for these experiments.

• **cAMP measurement** : Cyclic AMP levels were assessed in cultured human PASMCs using ELISA kit from R&D systems (Abingdon, UK). Cells were grown in DMEM/F12 containing 10% FBS to 70% confluence before being stimulated for varying times with treprostinil (TREP; 1 μM) or in combination with 1 μM cilostasole (PDE3 inhibitor), given 1 hr prior to treprostinil. Results are expressed as pmol of cyclic AMP per mg of total protein.

• **Cell proliferation assay** : Human PASMCs from IPAH patients were grown for 24 hrs in DMEM/F12 containing 10 % FBS and starved for 48 hr in media alone. Cells were then stimulated with 10% FBS ± treprostinil (1μM), cilostazole (1 μM or 10 μM) or in combination. Cells were counted after 4 days using ADAM-MC cell counter (Korea).

• **Myography** : To assess whether PDE3 inhibition could potentiate relaxation to treprostinil, rat tail arteries (~2mm long) were mounted onto an isometric myograph (500A JP Trading, Denmark). After a normalisation procedure vessels were contracted with 1 μM phenylephrine followed by sequential application of increasing doses of treprostinil ± cilostazole (10 μM).

RESULTS

1. Time course of cyclic AMP levels in IPAH cells

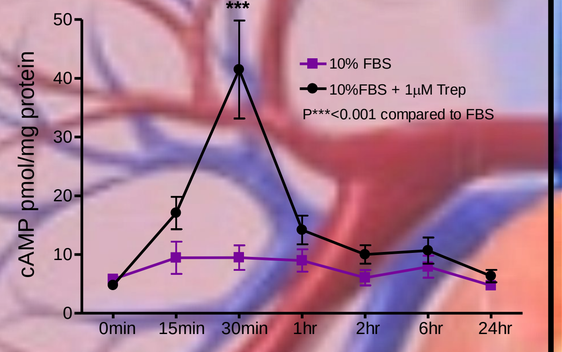

Figure 1. Cyclic AMP levels in human PASMCs in the absence and presence of treprostinil (1uM; TREP). Levels peaked within 30min and dropped back to control thereafter.

2. Effect of PDE3 inhibitor on cyclic AMP

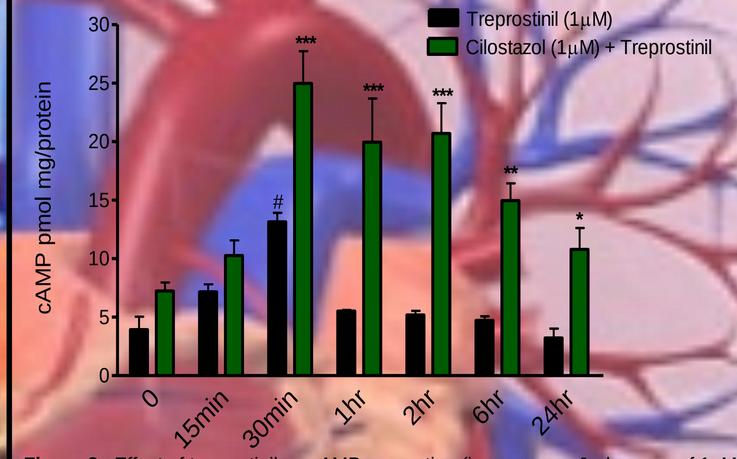

Figure 2. Effect of treprostinil on cAMP generation (in presence & absence of 1μM cilostazole) in distal human PASMCs derived from IPAH lungs

3. Effect of PDE3 inhibitor on cell proliferation

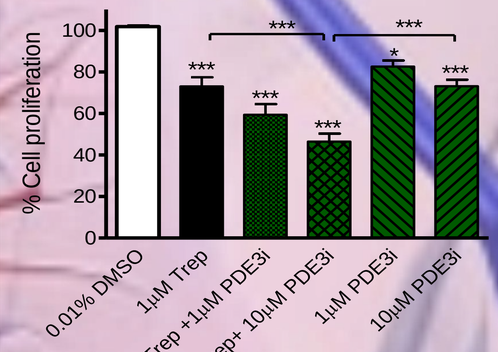

Figure 3. Cilostazole potentiates the anti-proliferative effects of treprostinil in distal human PASMCs derived from IPAH lungs

#=p<0.001 compared to control. *p<0.05, **p<0.01, ***p<0.001 with respect to the control

SUMMARY AND CONCLUSION

❖ Cyclic AMP levels peaked within 30mins of treprostinil treatment, but surprising dropped within an hour to almost the basal value. We hypothesize this is due to the higher activity of the phosphodiesterase enzymes which break down cAMP (PDE 1, 3, 4).

❖ High activity of PDE3 was assumed as we observed that the peak cAMP level was higher with the combination of treprostinil and the PDE3 inhibitor, cilostazole than with treprostinil alone and elevation was significantly sustained for up to 24 hrs compared.

❖ High activity of PDE3 might explain in part the ineffectiveness of prostacyclins (PGI_2) in long-term therapy and we propose that a PDE3 inhibitor might potentiate PGI_2 effects in PAH.

❖ In our study we demonstrated that cilostazole could significantly potentiate cAMP levels, antiproliferative and relaxation effects of treprostinil suggesting that this combination could be clinically beneficial for treating PAH.

❖ Cilostazol is already in clinical use for intermittent claudication (peripheral vascular occlusive disease) where it increases exercise tolerance and favorably modifies the plasma lipid profile, suggesting additional beneficial effects in diseases associated with atherosclerosis[2] and could be possibly a new therapeutic agent for IPAH.

4. Effect of PDE3 inhibitor on relaxation

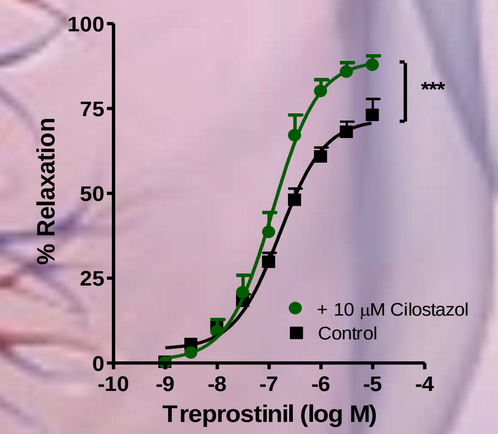

Figure 4. Cilostazole potentiates the relaxation response of treprostinil.

REFERENCES

1. Murray F, et al. *Am J Physiol Lung Cell Mol Physiol* 2007; 292: L294-L303.
2. Shakur Y, et al. Cardiovasc Drugs Ther. 2002 Sep;16(5):417-27