How do Riociguat and Sildenafil affect endothelial cells in Pulmonary Hypertension?

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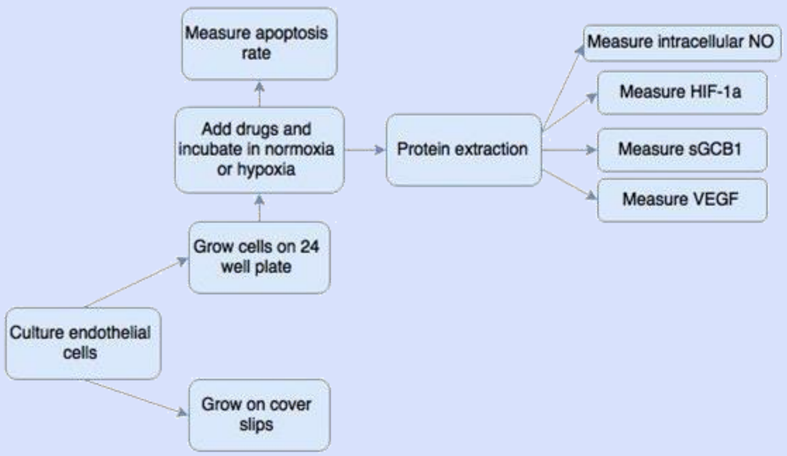
Introduction

- Pulmonary hypertension (PH) is a disease that arises from injury of the blood vessel linings in the lungs
- The causes high blood pressure in the lungs which can lead to right-side heart failure and premature death
- The pathways leading to injury of the endothelium have provided targets for drug therapies such as riociguat and sildenafil
- Early diagnosis and an appropriate therapy choice is vital for effective management of the disease (1)
- Drugs often show different results in each individual which is why personalised medicine approach would be more beneficial
- To be able to provide patients with the best treatment, we need to understand the mechanism of these drugs

Aims

To study the effect of PH drugs: riociguat and sildenafil on the endothelial cells isolated from blood from patients with PH which are known as blood outgrowth endothelial progenitor cells (EPCs) [1].
The following parameters are important markers for endothelial dysfunction in PH were therefore measured:
•Nitric Oxide (NO- blood vessel relaxant)
•HIF-1α (master regulator of response to low oxygen concentration)
•sGCβ1 (only known receptor of NO)
•VEGFR2 (receptor of protein involved in formation of angiogenesis)

Methods



Protein was extracted to carryout the following experiments:

- Western blot to assess and quantify HIF-1α, sGCβ1 and VEGFR2 levels with and without drugs under normoxic and hypoxic conditions
- VEGF and NO levels were measured using Enzyme-linked immunosorbent assay (ELISA)
- Immunoflourescence staining of HIF-1α was observed under confocal microscopy to determine its location (cytoplasmic or nuclear) in normoxia and hypoxia, and if the drugs had any effect on its location

Results - increase in nuclear HIF-1α during hypoxia

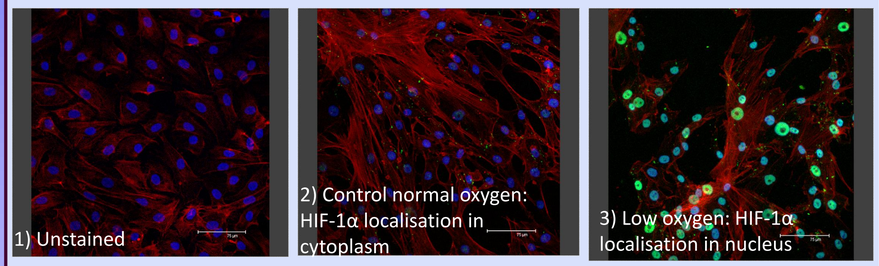
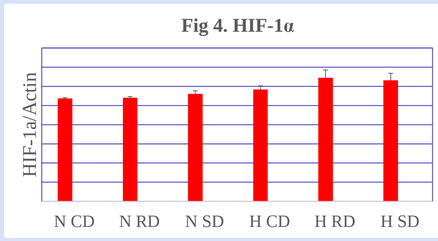
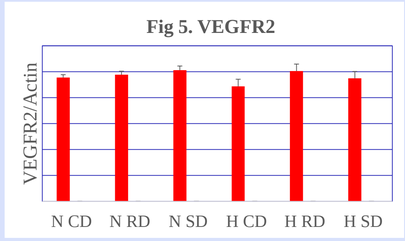
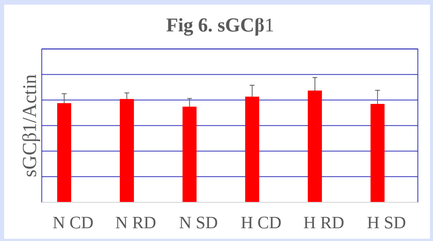


Fig 1, 2, 3. shows immunofluorescence staining of healthy pulmonary microvascular smooth muscle cells incubated in normoxia/hypoxia for 4 hours and stained with DAPI (nucleus), Phalloidin (cytoskeleton) and FITC (HIF-1α).

Results – western blot of HIF-1α, sGCβ1 and VEGFR2







Key:
N= normal O2 concentration
H= low O2 concentration
R= riociguat
S= sildenafil

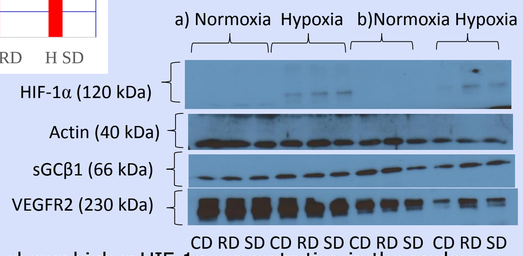
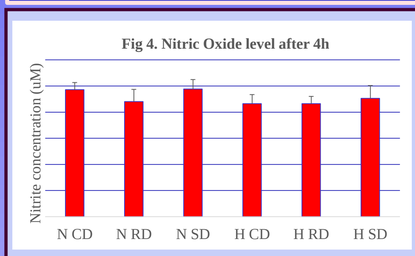


Fig. 4, 5 & 6. representative data shows higher HIF-1α concentration in the nucleus during after incubation in hypoxia

Results: lower NO level in hypoxia



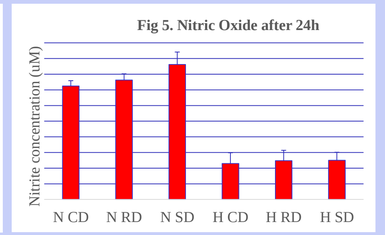


Fig. 7 & 8. a) Representative data showing lower NO level in hypoxia than normoxia in both durations, but more so in the 24h incubation
b) Increased NO level after 24h in both normoxia and hypoxia, with sildenafil showing a bigger increase across all data.

Discussion

- Higher level of HIF-1α was seen in PH endothelial cells which could indicate a dysfunction in the HIF-1α pathway
- Both riociguat and sildenafil slightly increased the level of intracellular NO. This will increase relaxation of blood vessels and alleviate PH symptoms
- Riociguat increased sGCβ1 level, increasing the sensitivity to NO and increased blood vessel relaxation.
- Lower level of VEGFR2 was seen in diseased cells but was resurrected by addition of drugs. This suggests that in PH the endothelial cells begin to lose their ability to repair and develop new blood vessels
- The drugs increased the level of VEGFR2 and therefore improves the ability for angiogenesis in the lungs
- From my findings, I can conclude that riociguat and sildenafil treat PH by increasing intracellular NO and VEGFR2 and increasing sensitivity of sGCβ1 to NO
- Future research could measure these protein levels using endothelial cells from healthy individuals as 'control' rather than from PH patients

Glossary

- Endothelial cells: *Layer of epithelium lining interior surface of blood vessels*
- Normoxia: *Normal oxygen level (21%)*
- Hypoxia: *Low oxygen concentration*
- Angiogenesis: *The development of new blood vessels*
- Phenotype: *Physical characteristics determined by genes*

Acknowledgements

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References

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2. Toshner M, Voswinckel R, Southwood M, Al-Lamki R, Howard LSG, Marchesan D, et al. Evidence of Dysfunction of Endothelial Progenitors in Pulmonary Arterial Hypertension. American Journal of Respiratory and Critical Care Medicine. 2009;180(8):780-7

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