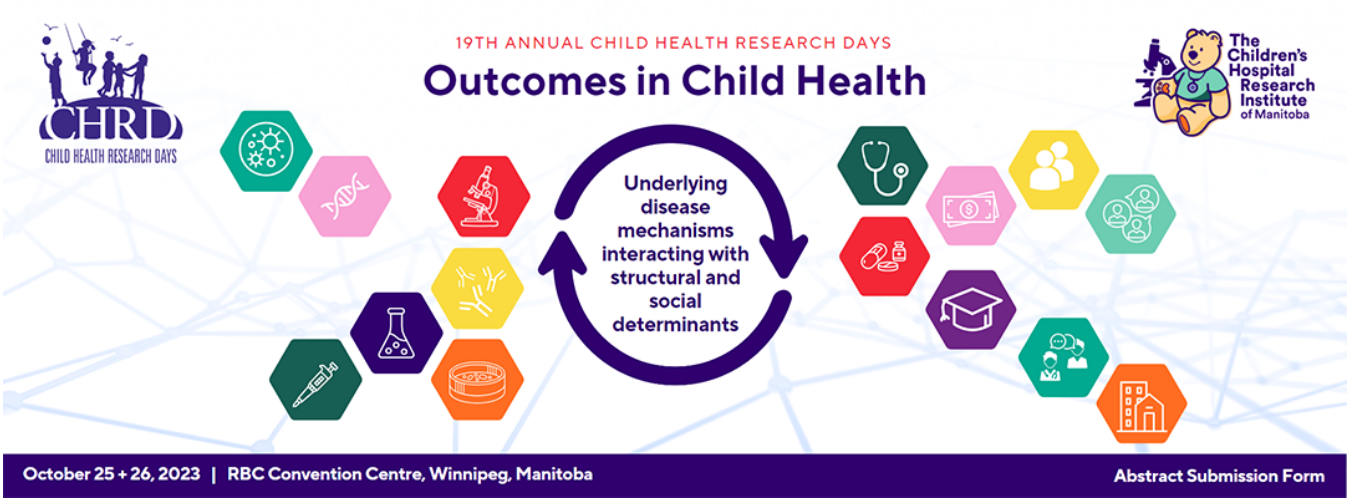

CHRD 2023: Abstract Submission Form

Submitter Name
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Presenter Status
PhD Student

Research Category
Basic Science

Role in the project
Design
Perform Experiments
Analyze Data
Write Abstract

Title
Discovery of A Novel and Selective Adenylyl Cyclase Isoform 6 Activator: A Potential Therapeutic Option for Pulmonary Hypertension in the Neonates

Background
Persistent pulmonary hypertension of the newborn (PPHN) is a failure of pulmonary vascular relaxation after birth. Adenylyl cyclase (AC) mediates vasorelaxation and is crucial drug target. AC isoforms 3, 6, 7 and 9 are expressed in neonatal pulmonary arteries (PA); AC6 is predominant. AC6 is inhibited due to S-nitrosylation in PPHN PA, and in PA myocytes exposed to hypoxia.

Objective
Analyze the activity of a series of novel forskolin derivatives against AC isoforms 3, 5, 6, 7, and 9.

Methods
Through homology modelling, the AC6 structural model was generated. Functions of forskolin-interacting residues of the AC6 allosteric pocket were discovered by ligand docking and using site-directed mutagenesis. HEK293T cells stably overexpressing AC isoforms 3, 5, 6, 7 or 9 were cultured in normoxia (21% O2) or hypoxia (10% O2) for 72 hours, or challenged with nitroso donor S-nitrosocysteine (CSNO), followed by AC activity assay. Forskolin-dependent real-time cAMP generation was measured using a live-cell cAMP biosensor. A library of forskolin derivatives was synthesized and screened against all AC isoforms. Dose-response testing was performed for compounds showing AC6 selectivity.

Results

Hypoxia and CSNO both decreased the catalytic activity of AC6 due to S-nitrosylation. Residues T500, N503, and S1035 interact with forskolin; mutation of T500, N503, or S1035 diminished forskolin-stimulated AC activity relative to AC6WT. Forskolin site mutants were not further inhibited by hypoxia or CSNO. cAMP dynamics showed forskolin unresponsiveness of all mutants. Among forskolin derivatives tested, forskolin 1α, 9α-carbonate offered relatively selective activation of AC6.

Conclusion
Although hypoxia inhibits AC6, forskolin can stimulate catalytic activity of AC6 under hypoxic conditions. The hypoxia inhibitory mechanism does not involve amino acids that interact with forskolin. Selective AC6 reactivation is a potential therapeutic target in PPHN. The C1 carbamate derivative of forskolin may interact selectively with the AC6 FSK binding site.

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