STRONG CHILDREN'S RESEARCH CENTER

Summer Research Scholar

Name: Olivier Charest

School: University of Wisconsin-Stout

Mentor: George A. Porter, Jr. M.D., Ph.D.

ABSTRACT

Title: Sildenafil Effects on Mitochondrial Function

Background:

Sildenafil (Sil) is a drug used as a pulmonary arterial vasodilator in infants and children with primary post-surgical pulmonary hypertension. It is hypothesized that Sil promotes the closure of the mitochondrial permeability transition pore (mPTP). Sil inhibits phosphodiesterase type 5, which is a key enzyme involved in the regulation of cGMP-specific signaling pathways in normal physiological processes such as smooth muscle contraction and relaxation. However, it is not known how Sil affects mitochondrial ATP generation through the ATP synthase -also called complex V- of the electron transport chain. One possible mechanism is that Sil inhibits the mPTP, a transmembrane pore located within the ATP synthase in the inner mitochondrial membrane.
To investigate the effects of Sil on heart mitochondria, we compared the effects of Sil to those of cyclosporin A (CsA), a known inhibitor of the mPTP. We measured mPTP-induced mitochondrial swelling and measured ATPase activity and the dynamics of complex V to form dimers and oligomers. In addition, we injected neonatal mice from postnatal days 1 through 6 with 10 µg/g Sil and performed echocardiography to measure cardiac function on day 7.

Objective:

- Examine the effect of Sildenafil on the mPTP
- Examine the effect of Sildenafil on ATPase activity
- Examine the effect of Sildenafil on the dynamic of Complex V to form dimers and tetramers
- Examine the effect of sildenafil on the heart morphology of neonatal mice

Results:

Sildenafil did not inhibit pore opening caused by $Ca2^+$; there was a significant increase in swelling for Sil/$Ca2^+$ compared to Ctr ($p=0.0002$) but no significant difference between $Ca2^+$ and Sil/$Ca2^+$ ($p=0.1828$).
Sildenafil alone did not reduce ATPase activity. Treatment with $Ca2^+$ and CsA/$Ca2^+$ greatly reduced ATPase activity but Sil/$Ca2^+$ did not, although this treatment did decrease in ATPase activity in a different in-gel ATPase assay ($n=2$)
CypD and ATP5A showed less expression in the complex V monomer for CsA/$Ca2^+$.
Sildenafil-injected neonatal mice had significantly reduced heart rate ($p=0.0482$) and cardiac output ($p=0.0104$) compared to vehicle-injected neonatal mice, but there was no change in stroke volume. Sildenafil-injected mice also had a significantly decreased body weight ($p=0.0004$) at P7, compared to vehicle-injected mice.

Conclusion:

- Sildenafil did not prevent Ca2+induced mitochondrial swelling attributed to opening of the mPTP.
- Sildenafil preserved ATPase activity, even in the presence of Ca^{2+}, in an enzymatic activity assay but did not maintain ATPase activity in the presence of Ca^{2+} in an in-gel assay for ATP synthase (n=2).
- Sildenafil-injected mice showed a significant decrease in heart rate and cardiac output, but their stroke volume was not affected.
- Sildenafil-injected mice had a significantly lower body weight at P7 compared to vehicle-injected mice.