Discovery and in vivo evaluation of novel inhibitors of the hydrolytic activity of mitochondrial F1Fo ATP synthase to alleviate myocardial ischemia reperfusion injury

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Background: F1Fo ATP-synthase is the mitochondrial complex responsible for ATP production. During myocardial ischemia, ATP synthase reverses its activity to hydrolyze ATP leading to energetic deficit and cardiac injury. We have previously discovered via in-silico virtual screening selective hydrolase inhibitors with pyrazole-pyridine scaffold and verified their inhibitory effect on hydrolysis on isolated murine mitochondria.

Purpose: In the present work we aimed to 1) synthesize novel hydrolase inhibitors, 2) select the best candidates to evaluate their effect against myocardial ischemia (I) reperfusion (R) injury in vivo and 3) investigate the underlying molecular mechanisms of cardioprotection.

Methods: The inhibitory effect on ATP hydrolysis for all compounds was evaluated on isolated murine heart mitochondria and at a cellular level on H9C2 cells in the presence of rotenone where their ability to maintain membrane potential was monitored. The previously described ATP hydrolase inhibitor BTB06584 (BTB) was used in vitro and in vivo as a reference and three novel compounds were selected for in vivo evaluation namely 1117, 1119 and STK-71. In the first cohort, C57Bl6 mice were randomized into 5 groups that received intravenously: 1) vehicle, 2) 1117, 3)1119, 4) STK-71 and 5) BTB. The administration was performed 10 min prior to 30 min of I and 2 hours of R and infarct size (IS) was determined. The dose of each compound was based on the effective concentration needed at a cellular level to inhibit the ATP hydrolysis. In a second cohort of mice, the best candidate and vehicle were given 10 min after the induction of I and IS was measured to test the intervention in a more translational setting. Finally, in the third cohort, the cardioprotective mechanism was investigated at the 10th min of R.

Results: We discovered three candidates that inhibit the ATP hydrolytic activity with comparable IC50 values to BTB. These three compounds inhibited ATP hydrolysis on the H9C2 cells at 50μM while BTB was inactive at the same concentration. In vivo, the three novel inhibitors reduced IS compared to the control group with STK-71 having the most remarkable cardioprotective effect while BTB did not result in attenuation of IS. STK-71 when given after the induction of I, also reduced IS indicating that its cardioprotective effect is pertained in a translational protocol. Regarding the mechanism of cardioprotection, STK-71 increased T173 phosphorylation and activation of PKA which led to the downstream activation of phospholamban. STK-71 also led to the significant activation of the RISK pathway, increased the expression of the antiapoptotic protein Bcl-Xl and reduced apoptosis.

Conclusion: We discovered a novel pyrazolopyridine derivative that acts as an inhibitor of ATP hydrolysis and reduces myocardial IS in vivo. Its cardioprotective mechanism involves the activation of PKA signaling and alleviation of apoptosis.