Inhibiting Pyruvate Kinase Muscle Isoform 2 with Shikonin Regresses Supra-coronary Aortic Banding induced Group 2 Pulmonary Hypertension

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Background
Group 2 pulmonary hypertension (PH), defined by mean pulmonary arterial pressure>20 mmHg with elevated pulmonary capillary wedge pressure>15 mmHg, is the most prevalent form of PH. There is no approved therapy for this condition and patients die from right ventricular (RV) failure. Metabolic dysregulation characterized by a biventricular pro-glycolytic shift caused by increased pyruvate kinase muscle (PKM) isozyme 2 to 1 ratio is believed be a key driver of RV failure. We hypothesized that shikonin, a PKM2 inhibitor, could improve RV function in a rat model of group 2 PH.

Methods
Group 2 PH was induced by supra-coronary aortic banding (SAB) in 5-week old male Sprague-Dawley rats. Echocardiography was performed 6-weeks post SAB surgery, to verify the presence of group 2 PH prior to randomization to placebo versus shikonin treatment. SAB and sham rats were administered shikonin (2 mg/kg/day) or DMSO (5%) intraperitoneally (IP) for 2-weeks. Post-treatment echocardiography was performed, followed by left and right heart catheterization and tissue collection. RV tissue was processed for histology analysis and Western blotting was performed to measure PKM2 and PKM1 expression.

Results
Pre-treatment echocardiography showed a marked reduction of tricuspid annular plane systolic excursion (TAPSE) (2.29±0.15 vs. 3.57±0.17 mm, $p<0.0001$) and pulmonary artery acceleration time (PAAT) (22.74±1.02 vs. 33.97±1.012 ms, $p<0.0001$), and increased RV free wall (RVFW) thickness (1.57±0.08 vs. 1.11±0.07 mmHg, $p=0.0009$) in SAB vs. sham rats. Shikonin-treated SAB rats had improved PAAT (32.1±1.29 vs. 22.1±1.24 ms, $p=0.03$) and a trend towards reduced RVFW (1.16±0.10 vs. 1.43±0.04 mm, $p=0.13$). Cardiac catheterization showed decreased right ventricular systolic pressure (RVSP) (31.53±0.92 vs. 55.65±1.87 mmHg, $p<0.0001$) in shikonin-SAB vs. DMSO-SAB rats. RV weight was decreased in the shikonin-SAB vs. DMSO-SAB rats (0.29±0.02 vs. 0.46±0.05 g, $p=0.01$). H&E staining showed decreased RV myocyte cross-sectional area in shikonin-SAB vs. DMSO-SAB rats (477±48.6 vs. 1136±66.23 μm^2, $p<0.0001$). Western blot showed a decrease in the RV PKM2/PKM1 expression ratio, related primarily to decreased PKM2 expression in the shikonin-SAB vs. DMSO-SAB rats (0.37±0.05 vs. 1.47±0.14, $p<0.0001$) (See Figure).

Conclusion
Two-week treatment of SAB rats with shikonin significantly reduced the severity of SAB-induced Group 2 PH, evident as reduced RVSP and RV hypertrophy. Shikonin restored the normal PKM2 to PKM1 ratio. Our data suggest that an increased in PKM2/PKM1 ration in the

RV is maladaptive and suggests that, shikonin, a widely consumed Chinese herbal remedy, may be a novel therapeutic agent for group 2 PH.