

Calpain Inhibition Reduces Myocardial Cellular Injury and Myocardial Stunning During Hypoxia And Reoxygenation Associated With Cardiopulmonary Bypass

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Objective: Congenital heart defects often cause hypoxic conditions in pediatric patients. Cardiopulmonary bypass (CPB), used in the repair of these defects, can result in myocardial injury and stunning due to reoxygenation injury. The inhibition of calpain, a ubiquitous calcium ion dependent cysteine protease, has been shown to inhibit cardiopulmonary injury. The hypothesis is that calpain inhibition will reduce injury after hypoxia and reoxygenation.

Methods: Crossbred piglets (5-7 kg) were placed under hypoxic conditions ($\text{FiO}_2 = 12\%$) for 90 minutes. Three piglets were administered calpain inhibitor (*carbobenzoxy-leucinyl-leucinyl-tyrosine-fluormethyl ketone*; 1mg/kg, IV) 20 min. prior to reoxygenation on CPB. Six piglets were administered saline as a control. Following this the piglets were reoxygenated on CPB for 60 minutes. After CPB, piglets were allowed to recover for 120 minutes on ventilation ($\text{FiO}_2 = 42\%$).

Results: Intact LV troponin I levels were maintained in the calpain inhibitor treated group compared to controls ($65 \pm 6.5\%$ vs. $53 \pm 1.3\%$; $p < 0.05$). Troponin I 26 kDa degradation product (treated $16.6 \pm 6\%$ vs. controls $26.8 \pm 2.3\%$; $p < 0.05$) and 15 kDa degraded fragment (treated $8.8 \pm 1.5\%$ vs. controls $11.35 \pm 4.3\%$; $p < 0.05$) were lower in the calpain inhibitor treated group. LV calpain activity was lower in the calpain inhibitor treated group compared with controls (195.6 ± 10.2 vs. 234.3 ± 17.4 , fluorescent units, respectively; $p < 0.05$). Western blot analyses of LV protein demonstrated calpain I (0.09 ± 0.01 vs. 0.09 ± 0.03 , ratio of calpain to GAPDH; $p < 0.05$) and calpastatin ($0.18 \pm .04$ vs. $0.18 \pm .08$, ratio of calpastatin to GAPDH; $p < 0.05$) levels did not differ between treatment animals and controls. Pulmonary vascular resistance of calpain animals to controls was not improved at 120 minutes of recovery (338 ± 13.8 vs. 260 ± 264 dyne*s/cm⁵, respectively.) Left ventricular change in pressure over time for calpain treated versus untreated animals was similar at 120 minutes of recovery (661 ± 343 vs. 892 ± 191 mmHg/s, respectively.)

Conclusions: Calpain inhibition appears to preserve troponin I from degrading during reoxygenation associated with CPB. Pulmonary dysfunction and hypertension was not improved however. Left ventricular systolic pressure and pulmonary vascular resistance were not improved compared to the untreated group. While calpain inhibitor preserved intact troponin I levels, which may indicate reduced myocardial stunning, cardiopulmonary function was not improved with calpain inhibitor administered only during reoxygenation.