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**Objectives** Calpains have been implicated in myocardial ischaemia-reperfusion (I/R) injury. The mitochondrial permeability transition pore (mPTP) subsequently triggers apoptotic cell death during I/R. However, the mechanistic link among calpain activity, mPTP opening and apoptosis in myocardium during I/R remains to be elucidated.

**Aim** This aim of this study was to investigate whether the activation of calpain in I/R cardiomyocytes is associated with alterations in mPTP and subsequent apoptotic cell death.

**Methods** Primary cultured neonatal mouse cardiomyocytes were deprived of oxygen and glucose to simulate ischaemia, and restored oxygen and sugar supply to simulate reperfusion (simulated I/R injury). To determine the influence of calpain activity on mPTP and apoptosis, cells were pretreated with PD150606 (PD, a specific calpain inhibitor). Apoptosis in cardiomyocytes was determined by TUNEL-staining and caspase-3 activity analysis. To identify the activated calpain isoform implicated in myocardial I/R injury, the autolysis of the N-terminal domains of the catalytic subunit of m- and m-calpain in cardiomyocytes were detected by immunoblot analysis. mPTP opening in cardiomyocytes was assessed by using the calcein–cobalt method and mitochondrial membrane potential ($\Delta$ym) by imaging cells loaded with JC-1.

**Results** Reperfusion following ischaemia, rather than ischaemia, led to the autolysis of the N-terminal domains of the catalytic subunit of m-calpain in cardiomyocytes. However, the autolysis of the N-terminal domains of the catalytic subunit of m-calpain in cardiomyocytes was not observed in the investigation. The percentage of TUNEL-positive cardiomyocytes and caspase-3 activity was significantly less in the PD (20.38%±2.23% and fold of changes, 1.43±0.13) compared with the untreated I/R (31.48% ±1.65% and fold of changes, 2.21±0.17) group. Moreover, pD pretreatment of cardiomyocytes dramatically suppressed the opening of mPTP and the loss of $\Delta$ym caused by I/R.

**Conclusions** Myocardial I/R can lead to the activation of m-calpain, which subsequently triggers apoptotic cell death by inducing mPTP opening.