

AUGMENTATION OF CREATINE KINASE *IN VITRO* PROTECTS AGAINST SIMULATED ISCHAEMIA REPERFUSION INJURY

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Creatine kinase (CK) catalyses the interchange of high energy phosphates to buffer ATP levels and maintains cellular energy homeostasis. The heart expresses three isoforms: sarcomeric mitochondrial CK (CKMT2), and the cytoplasmic CKM and CKB isoforms which form homo (MM/BB)- and hetero (MB)-dimers. Impaired CK activity is associated with heart failure and increases susceptibility to ischaemia/reperfusion injury.

We hypothesised that augmentation of CK isoenzymes *in vitro* would improve cell viability following exposure to hypoxia/reoxygenation. For this purpose we created CK overexpression systems by cloning the open reading frame of the different CK isoform sequences into pcDNA3.1 expression vector and stably selected and characterised overexpressing HEK293 cell lines.

The generated cell lines displayed increased CK activity in addition to individual CK isoenzyme activities. CKMT2, CKM and CKB cells had elevated total CK activity ($P<0.001$; $P<0.001$; $P<0.01$ One-way ANOVA, Dunnett's post-test vs HEK293). Furthermore immunocytochemistry showed that CKMT2 co-localises with mitochondrial marker COXIV in the intermembrane space following transient transfection in HL1 atrial cell line.

Both stable and untransfected HEK293 cells were exposed to simulated ischaemia/reperfusion by incubating at 1% O₂ for 18 h, followed by re-oxygenation at 95% O₂ for 2h. The positive control rapamycin was supplemented into the cell media 4 hours prior to hypoxia. Viability analysis by propidium iodide detection using a CyAN flow cytometer at 488nm, showed increased cell survival by 33% in CKMT2, 47% in CKM and 58% in CKB cells when compared to untransfected HEK293 controls (in all cases $P<0.05$, One-Way ANOVA Dunnett's post-test vs HEK293).

To determine whether protection was due to changes in antioxidant capacity, cells were loaded with the reactive oxygen species indicator dye, DCFH₂-DA, and exposed to H₂O₂-induced oxidative stress. Overexpression of CK isoenzymes failed to attenuate fluorescence from oxidised dye in contrast to the known antioxidant, Trolox. Transient expression of CK constructs in the HL1 cell line was used to test the effects of anthracycline exposure on cell viability (48 h doxorubicin). Pre-treatment with Trolox increased cell survival by 12.4% (79.4% \pm 2.0 vs. 67% \pm 1.5 in empty-vector control cells; $P<0.01$) whereas overexpression of CK isoenzymes did not alter cell death rates.

In conclusion, overexpression of any one (of three) cardiac creatine kinase isoenzymes protects against ischemia/reperfusion *in vitro*. This most likely reflects enhanced energy reserve due to elevated CK activity, since response to oxidative challenge was unaltered. Further mechanistic studies and *in vivo* confirmation of these findings are merited.