Effect of acupuncture intervention on the changes of cytoplasmic and mitochondrial Ca\(^2+\) concentration following eccentric contractions in rat skeletal muscle

Aishan Liu, Fangming Liu, Xuelin Zhang, Yarong Wang, Mei Kong, Xiang Zhang, Meiling Ye
Qufu Normal University

Objective
The purpose of this study was to evaluate the effect of acupuncture intervention on the changes of cytoplasmic and mitochondrial Ca\(^2+\) concentration following eccentric contractions (ECC) in rat skeletal muscle.

Methods
24 healthy male Wistar rats were randomly divided into 4 groups: control group (C, n=6), electrical stimulation group (E, n=6), electrical stimulation group with acupuncture intervention (EA, n=6), electrical stimulation group with acupuncture + TRP channel inhibitor Gd\(^{3+}\) (EAI, n=6). The animal model of eccentric induced skeletal muscle injury was established by electrical stimulation on spinotrapezius muscle of anaesthetised rats in vivo, that is to say, the intact spinotrapezius muscle of adult Wistar rats was exteriorized, and tetanic eccentric contractions (100 Hz, 10 sets of 50 contractions) were elicited by electrical stimulation during synchronized muscle stretch of 10% resting muscle length. Cytoplasmic Ca\(^2+\) accumulation were determined by loading the muscle with fura 4-AM using fluorescent imaging in vivo, and mitochondrial Ca\(^2+\) concentration were determined by loading the muscle with fura 2-AM using fluorescent imaging in vitro, and recorded changes of muscle maximum tetanic force.

Results
(1) In vivo, compared with the C, cytoplasmic Ca\(^2+\) accumulation increased more rapidly during ECC in the E (P < 0.001). Acupuncture intervention significantly reduced cytosolic Ca\(^2+\) accumulation in the EA compared with the E (P < 0.01), and we discovered that muscle deformation generated by acupuncture intervention induced a robust Ca\(^2+\) spark response confined in close spatial proximity to the sarcolemmal membrane in intact muscle fibers. Although no significant differences between the EA and EAI, Gd\(^{3+}\) abolished the majority cytoplasmic Ca\(^2+\) accumulation decrease during ECC in the EAI and a robust Ca\(^2+\) spark response disappeared compared with the EA.
(2) In vitro, compared with the C, mitochondrial Ca\(^2+\) concentration did not elevations in MCC in the E. EA cytoplasmic Ca\(^2+\) increased rapidly above the C and E (P < 0.01), respectively, but EAI significantly attenuated the increases in mitochondrial Ca\(^2+\) concentration compared with the EA (P < 0.01). (3). Compared with the C, maximum tetanic force was significantly lower in the E after ECC (P < 0.01). EA maximum tetanic force was significantly lower in the E after ECC (P < 0.01), but EAL abolished the majority maximum tetanic force increase after ECC (P < 0.05).

Conclusions
(1) Eccentric contraction caused cytoplasmic Ca\(^2+\) accumulation, but mitochondrial Ca\(^2+\) concentration decrease.
(2) Acupuncture can effectively reduce cytosolic Ca\(^2+\) overload, following by mitochondrial Ca\(^2+\) concentration increase, which in turn abnormally high cytoplasmic Ca\(^2+\) levels are buffered by the mitochondria, and improved muscle function, and the effect was associated to the TRP channels.