Acute lethal crush-injured rats can be successfully rescued by a single injection of high-dose dexamethasone through a pathway involving PI3K-Akt-eNOS signaling

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Method 1: Experimental design

On reperfusion following ischemia, the vascular endothelium becomes swollen and the vessel lumens are plugged and packed with erythrocytes, leukocytes, and platelets, resulting in the occurrence of a no-reflow phenomenon [1]. However, if the duration of ischemia is not long enough to cause no-reflow phenomenon, cytosolic toxic mediators including myoglobin derived from lysed skeletal muscle cells are released to the circulation following reflow phenomenon, leading to systemic inflammations with high mortality. However, the shorter ischemia duration (less than 4 hours in this model) is, the less cell damages are, resulting in less systemic inflammations with low mortality. We previously reported that a 5-hour bilateral hind limb ischemia exhibited the highest mortality rate (75%) at 24 hours after reperfusion compared with other durations of ischemia (0% mortality in less than 4-hour ischemia and only 10% mortality in more than 6-hour ischemia) [2], thereby providing a critical time window to cause reflow and rhabdomyolysis leading to serious CS.

Based on these reports, a 5-hour compression by the tourniquet was chosen for the crush interval in the current study [2,3] which provides a more practical animal model closely similar to lethal human CS than other animal models.

Method 2: Histology

All sections from tissues were scored by a blinded observer according to the semiquantitative histological scoring systems of skeletal muscle, lung and kidney described in the previous reports [4,5,6].

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