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The study of acupuncture intervention on the dynamic changes of Ca^{2+}, Na^{+}, H_2O_2 flux at earlier time points of rat skeletal muscle regeneration following eccentric exercise

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Objective In this study, the Non-invasive Micro-test Technique was adopted to study the dynamic changes of Ca^{2+}, Na^{+} , H_2O_2 flux during the early phase of skeletal muscle regeneration (0–24 h post-injury) after acupuncture intervention, and to explore the role of interaction between TRP channel and NADPH oxidase 2(NOX2) in the acupuncture mechanism.
Methods 324 healthy male Wistar rats were randomly divided into 6 groups: blank control group (C), electrical stimulation group (E), electrical stimulation group with acupuncture intervention (EA), electrical stimulation gropu with acupuncture +TRP channel inhibitor (EAT), electrical stimulation gropu with acupuncture + NOX2 inhibitor (EAN), electrical stimulation gropu with acupuncture + placebo (EAP). Except for group C, the animal model of eccentric induced skeletal muscle injury was established by electrostimulation on gastrocnemius of anaesthetised rats in vivo.Immediately after electrical stimulation, $GdCl_3$, apocynin and PBS buffer were injected by tail vein in EAT, EAN and EAP respectively. After 30 min, gastrocnemius muscle belly were stuck with acupuncture needles (diameter of 0.13 mm) in EA, EAT, EAN and EAP respectively. Shortly afterwards, a special polypropylene ring-shaped perforated vessel wall was sutured to the exposed gastrocnemius muscle, and to measure Ca^{2+}, Na^{+}, H_2O_2 fluxes by non-invasive micro-test technique in the phase of retaining needle, needle drawing immediately, 3h, 6h and 24h, respectively. The phase and time of detection in the C and E groups were consistented.
Results 1 When the gastrocnemius muscle was in a resting state, Ca^{2+} and Na^{+} were influx in small amounts, and H_2O_2 had a small eflux.
2 Effect of eccentric Exercise and acupuncture on the dynamic changes of Ca^{2+} flux at different phases : ① In the E, a small eflux occurred at 0min, 10min and 3h, and the eflux suddenly increased significantly at 6 h (p<0.05), followed by a small eflux at 24h; ② In the EA, a small eflux occurred during retaining needle and needle drawing immediately, and Surprisingly, a small influx was observed at 3h. After that, the eflux increased suddenly at 6h and 24h, and the eflux peaked at 24h, which was significantly different from the E group at 24h. (p<0.05); ③ The EAT showed a significant influx trend. Specifically, except a small eflux in the retention period and 3h phase, significant influx occurred immediately after the needle pulling、6h and 24h. Compared with C and EA, there was no statistically significant difference in net flux (influx and efflux), but Ca^{2+} oscillation amplitude (influx and eflux fluctuation amplitude) in EAT was significantly increased (p<0.001, p<0.01, respectively); ④ In EAN, the eflux was dominant. Specifically, there was significant influx in the retention period and immediately after needle pulling, and suddenly significant eflux was observed at 3h and 6h. The 6h phase was significant difference than that of E (p<0.05), afterwards, the eflux was significantly decreased at 24h, and was significantly different from 6h (p<0.05); ⑤ The EAP flowed outward at all phases, and the overall trend was similar to the E group. The eflux peaked at needle drawing immediately, which was significantly different from that of the concurrent phase E and EA (p<0.05), and the eflux was significantly decreased at 24h.
3 Effect of eccentric exercise and acupuncture on the dynamic changes of Na^{+} flux at different phases : ① In the E, the eflux occurred at during retaining needle and needle drawing immediately, and the influx occurred suddenly at 3h and 6h. There was a significant difference between 6h and the C

(p<0.001), and the eflux again occurred at 24h;② *In the EA, the flux occurred during retaining needle and needle drawing immediately, and after that, efflux occurred at the 3h, 6h, and 24h;*③ *The EAT efflux at all phases was in line with the trend of changes in the E.* ④ *T*he EAN only eflux at 6h (p<0.05), while the rest of the phases flowed inward, and the influx peaked at 3h, which was significantly different from that at needle drawing immediately (p<0.05). The influx amplitude decreased at 24h, showing a significant difference from 3h (p<0.01). ⑤ *The EAP only flowed inward at 3h, and the rest of the phases flowed outward.*

4 Effect of eccentric exercise and acupuncture on the dynamic changes of H_2O_2 flux at different phases : ① In the E, the influx occurred only at 10 min, and the rest of the phases flowed outward with an increasing trend. The peak value were reached at 24h, showing a significant difference with C group and 0 min(p<0.01), and extremely significant with 3h (p<0.001) and 6h (p<0.05);② EA only flowed inward during retaining needle, all other phases flowed outward and peaked at 24h, but the eflux was less than that of E and there was a significant difference at 6h with E (p<0.05);③ The EAT group flowed outward at all phases and reached the peak at 3h, showing a significant difference compared with the E and EA at the same phases (p<0.001); ④The EAN flowed outward at each time phase and peaking needle drawing immediately, but the flow velocity was higher than that of the EAT. There was an extremely significant difference compared with EA at the peaking phase (P<0.001) and a significant difference with EAT at the same phase (p<0.05);⑤ In the EAP, all phases flowed outward, but the flow rate was less than the EAT and EAN.

Conclusions 1 In the early subsequent phase of skeletal muscle regeneration, Ca^{2+} efflux decreased, while Na^{+} influx increased, accompanied by increased H_2O_2 efflux. 2 Acupuncture intervention increased Ca^{2+} efflux in the early subsequent phase of skeletal muscle regeneration and advanced the Na^{+} influx phase, with the decrease of H_2O_2 efflux, and the effect was related to the interaction of TRP channels synergize with NOX2 Activity.