The CB1 antagonist rimonabant improves skeletal muscle regeneration via fibrotic, necrotic and myogenic mechanisms in mice

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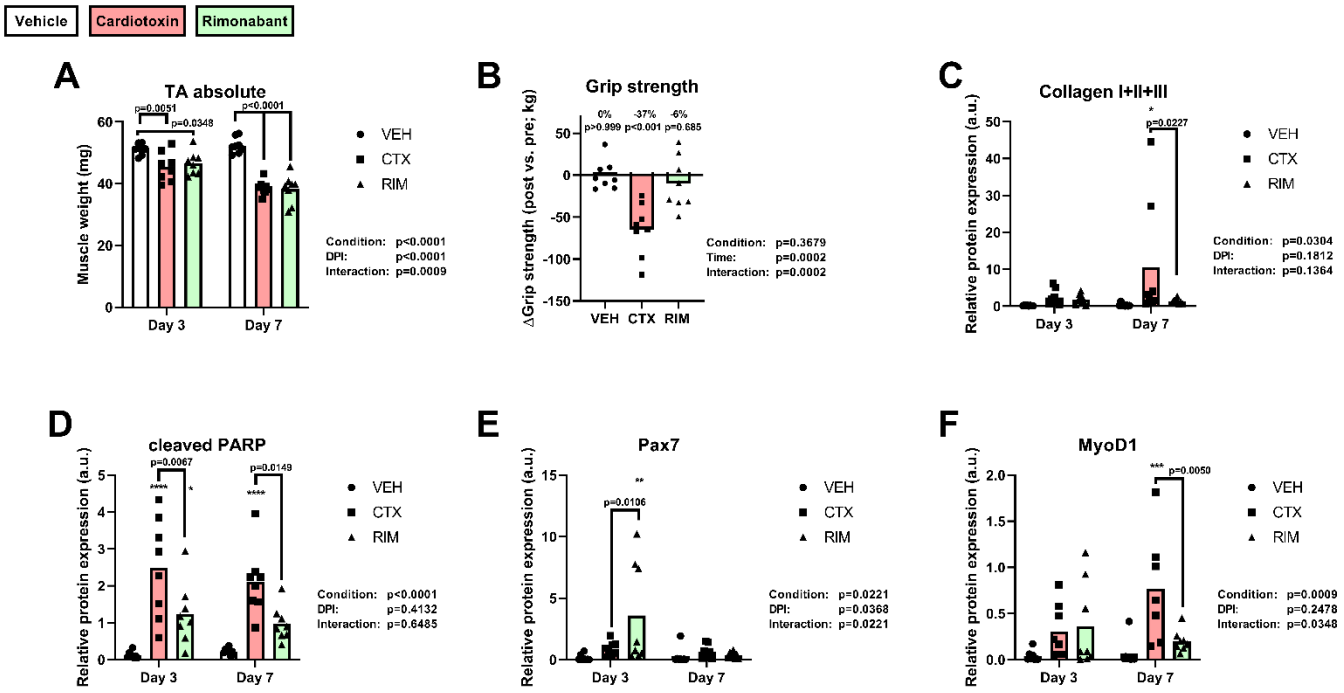
Background: Skeletal muscle (SkM) tissue is indispensable for energy metabolism and locomotion. Upon injury, an inflammatory and myogenic interplay is required for complete recovery. In patients and older adults, an imbalance between pro- and anti-inflammatory signaling and an impaired myogenic capacity of (stem) cells result in incomplete SkM recovery. This can result in tissue fibrosis, loss of SkM functionality and, eventually, disability. Therefore, novel targets that improve SkM regeneration are definitely required. It was shown that antagonism of cannabinoid receptor 1 (CB1) reduces fibrosis in heart tissue[2], stimulates *in vitro* myogenic capacity[3] and attenuates inflammation in dystrophic SkM[3], but it was never studied whether it can also improve *in vivo* regeneration of non-dystrophic SkM.

Aim: Therefore, the aim is to investigate whether, and via which mechanisms, the CB1 antagonist rimonabant improves SkM regeneration upon cardiotoxin (CTX)-induced injury.

Methods: Mice (n=48) were randomised into 3 conditions: controls (CON; n=16), cardiotoxin (CTX; n=16) and CTX + rimonabant (RIM; n=16). Mice received one saline (CON) or CTX injection (10μM; 30μl; CTX & RIM) in the m. Tibialis Anterior. Half of the CTX-injected mice were daily treated with rimonabant (10mg/kg/d; RIM), whereas the other half of the CTX group and the CON group were treated with vehicle. Within each condition, half of the mice were sacrificed 3 (n=8/condition; 3dpi) or 7 (n=8/condition; 7dpi) days post-injury. SkM functionality was assessed via grip strength, and SkM was analysed for fibrosis, necrosis and myogenicity. A two-way ANOVA analysis (condition*time) was applied.

Results: Whereas SkM mass was lower in CTX (3dpi: -12% & 7dpi: -30%) and RIM (3dpi: -9% & 7dpi: -31%) compared to CON (Fig.1A), the strength loss (5dpi) was larger in CTX than in RIM and CON (Fig.1B). On a molecular level, rimonabant treatment decreased CTX-induced upregulation of the fibrotic marker collagen (7dpi: -156%; Fig.1C) and of the necrotic markers cleaved caspase-3 (7dpi: -174%; $p_{bonferroni}$=0.004) and PARP (3dpi: -67% & 7dpi: -74%; Fig. 1D). Finally, the myogenic program was also affected by RIM, as the proliferation marker Pax7 was more expressed (3dpi: +120%), whereas the differentiation marker MyoD1 was less expressed (7dpi: -117%) compared to CTX (Fig.1E,F).

Conclusion: This study demonstrates that treatment with the CB1 antagonist rimonabant is a novel, effective strategy to improve SkM regeneration via fibrotic, apoptotic and myogenic mechanisms. This is particularly relevant for older adults or patient populations suffering from impaired regenerative capacity, such as COPD, type 2 diabetes and osteoarthritis.



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