INTRAVENOUS TREATMENT USING A NOVEL MURF-1 INHIBITOR (MYOMED #205) MITIGATES DIAPHRAGM WEAKNESS INDUCED BY MECHANICAL UNLOAD: A PRELMINARY DOSE-RESPONSE STUDY

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Several clinical conditions involving reduction of mechanical load (even within short-time periods e.g., mechanical ventilation, denervation) cause diaphragm's weakness and atrophy. The increased proteolytic activity of the ubiquitin-proteasome system is a key mechanism involved in this process and the E3-ligase MuRF1 (Muscle-ring finger protein 1) represents a crucial element of this proteolytical system. This study aimed to establish the dose-response profile of the MuRF1 inhibitor Myomed #205 (intravenous administration) by addressing diaphragm's contractile force after 12h of mechanical unload induced by 12h of unilateral denervation.

For the dose-response experiments, Wistar rats (~3 months old, male) were divided into 9 groups, 3 controls: 1) Sham 12h; 2) Denervated 12h (DNV 12h); DNV 12h + vehicle solution (VEH 12h) and 6 experimental groups which different doses of the Myomed #205 were tested: 4) DNV 12h + 12,5mg/kg; 5) DNV 12h + 25mg/kg; 6) DNV 12h + 50mg/kg; 7) DNV 12h + 100mg/kg; 8) DNV 12h + 150mg/kg and 9) DNV 12h + 250mg/kg (n=4/group). Rat's diaphragm unilateral paralysis was induced through a unilateral phrenicotomy. Sham animals underwent a mimetic surgical procedure. Sham 12h and DNV 12h groups were only subjected to diaphragm hemidenervation for 12h to detect the short-term impacts of mechanical unload on the diaphragm's contractile force. The other groups were treated by intravenous infusion (2mL/dose, 4 doses, 3h/3h) in Rat's tail vein with the vehicle solution that consists of DMSO (20%), PEG 400 (50%), and physiological saline 0.9% (30%) or the Myomed #205 solution (vehicle solution + #205) with the respective doses. After 12h, the diaphragm muscle was removed for *in vitro* contractility force measurements (AVS instruments). For statistical analysis, unpaired t-test or one-way ANOVA followed by Tukey's post hoc test were used for comparisons between two and three or more groups, respectively (GraphPad Prism v.8). Ethical committee approval CEUA ICB/USP 8728030320.

In vitro contractility measurements showed that 12h of hemidenervation significantly reduces diaphragm force compared to the Sham group (Sham 20±3.3 vs DNV 9±3.8 N/cm^2; ~55%, $p<0.05$). Intravenous infusion of the vehicle solution (VEH 12h) did not impact diaphragm force compared to DNV 12h group (DNV 9±3.8 vs VEH 10±2.5). Our results revealed that Myomed #205 at 50mg/kg was able to mitigate the diaphragm's maximal force loss 12h post denervation (VEH 10±2.5 vs Myomed #205-50 15.8±3.0 N/cm^2; ~30%, $p<0.05$), revealing a short-time protective effect. All the other doses employed were not effective in significantly protecting against force loss induced by denervation.

Overall, these results suggest that the Myomed #205 compound (50mg/kg) significantly mitigates diaphragm contractile weakness induced by 12h of denervation.

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