BIO103 demonstrates sharp functional improvement in an animal model of hindlimb immobilization

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Introduction

About Sarconeos: Sarconeos is a first-in-class drug candidate based on the activation of the MAS receptor (a major player of the alternative renin-angiotensin system) improving muscular anabolism, inhibiting myostatin, and that had demonstrated meaningful activity in animal models of muscular dystrophies. Sarconeos is being developed for the treatment of sarcopenia, an age-related degeneration of skeletal muscle, leading to loss of mobility in elderly people. In a Phase I clinical trial (SARA-PK), Sarconeos showed favorable pharmacokinetics and pharmacodynamics profiles. It is currently entering a clinical Phase 2b trial named SARA-INT. BIO101 is the active principal ingredient of Sarconeos.
BIO103 is a BIO101-derived hemisynthetic molecule.

Objectives: Skeletal muscle atrophy is a serious concern for patients afflicted by limb restriction due to surgery, several articular pathologies, or simply following cast immobilization.
Although disuse atrophy and sarcopenia share a common trait in loss of muscle mass, there are distinct differences in their wasting outcomes as well as in the biochemical processes that promote them.
The aim of this study was to characterize the impact of BIO103, a BIO101 hemisynthetic derivative, on muscle quality and function in an experimental model of disuse atrophy.

Methods

10 week-old C57BL6 female mice were treated orally either with vehicle or BIO103 at 50mg/kg*day under normal diet throughout a 14-day hindlimb immobilization phase and for 2 weeks during a remobilization phase.

Hindlimb immobilization model:
Hair on the right lower hindlimb was removed with clippers, the skin swabbed with 70% alcohol, and the hindlimb wrapped with a single layer of surgical tape.
A small amount of generic superglue was applied to the tape and a 1.5 ml plastic microfuge tube without the lid was placed over the leg, maintaining the foot in a dorsi-flexed position.

Histological studies: TA muscles of mice were frozen, serial-sectioned (7µm) and then stained using hematoxyline eosin. Anatomopathological study of the sections was performed. The lesional profile of each section was determined.

Muscle force measurement: *Tibialis anterior* distal tendon was attached to a force recorder. The sciatic nerve was stimulated by a bipolar electrode (10 V, 0.1 ms, 75-150 Hz, duration of 500 ms).

Gene expression: gene expression of selected markers was evaluated by semi-quantitative PCR. TA muscle RNAs were extracted, purified and converted into cDNA to allow gene expression analysis.

Results (I)

▪ Histological analysis of TA muscle

- Cross-sectional area

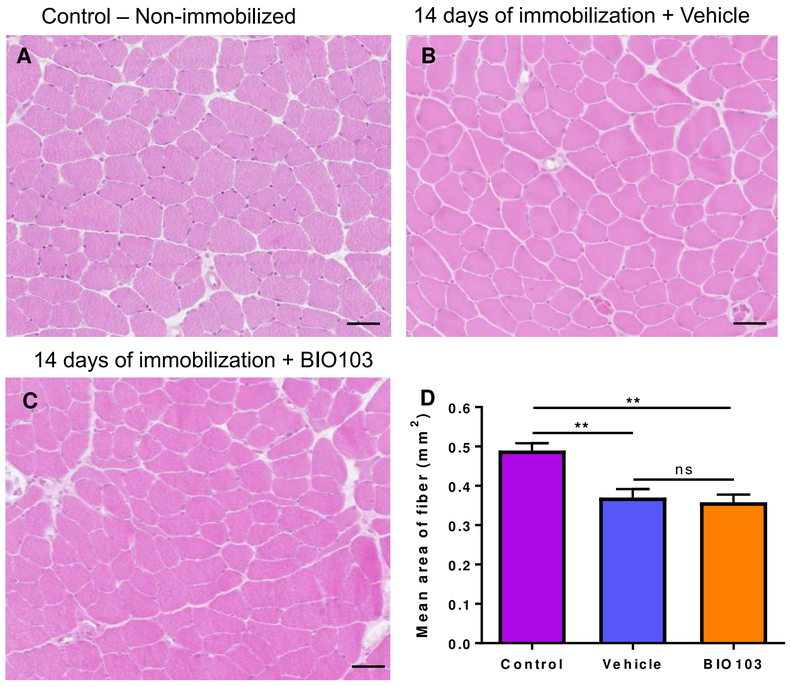


Figure 1: Hematoxilin/Eosin coloration of *Tibialis anterior* muscle sections reveal that after 14 days of immobilization, C57Bl6 vehicle-treated or BIO103-treated TA muscles (**B** and **C**) show no lesions compared to non-immobilized TA muscle (**A**), Nevertheless, TA fibers cross-sectional area in 14-day immobilized mice are significantly decreased in mice treated by vehicle or BIO103 (-24.3% and -26.8% respectively, p<0.01) (**D**).

→ BIO103 does not prevent the decrease of TA fibers cross-sectional area after 14 days of hindlimb immobilization

- Fiber size distribution

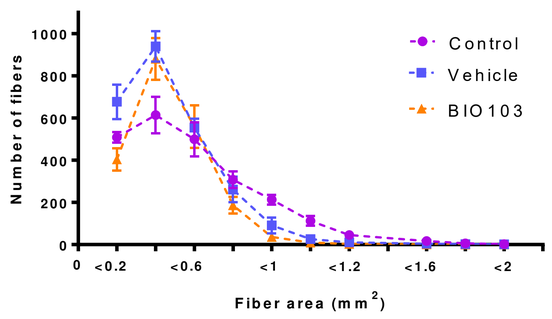


Figure 2: Fiber size distribution of TA muscle is slightly modified after 14 days of immobilization in vehicle and BIO103-treated muscles compared to non-immobilized control mice. Indeed, TA muscle presents a greater proportion of small fibers (surface comprised between 0.2mm² and 0.4mm²) whereas non-immobilized muscle present larger fibers (surface up to 1mm²), There is no difference in fiber size distribution between vehicle- and BIO103-treated mice.

→ BIO103 treatment has no impact on fiber size distribution after 14 days of hindlimb immobilization.

▪ Muscle weight

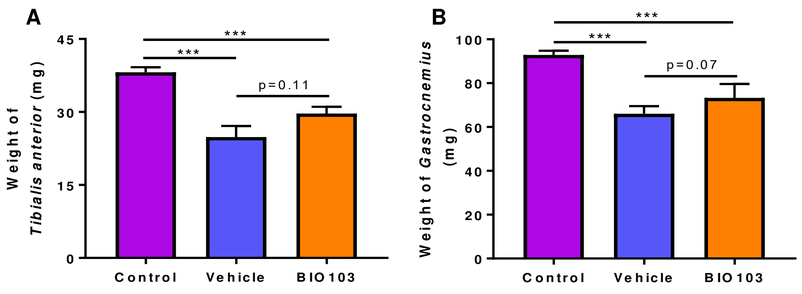


Figure 3: *Tibialis anterior* (**A**) and gastrocnemius (**B**) weight are significantly decreased after 14 days of immobilization in vehicule-treated and BIO103-treated mice (p<0,001). BIO103 treatment slightly tends to increase TA and gastrocnemius weight compared to vehicle-treated muscles but this difference is non-significant (p=0,11 and p=0,07 respectively).

→ BIO103 does not significantly increase TA or gastrocnemius weight after 14 days of hindlimb immobilization.

Results (II)

▪ *In situ* functional study

- Maximal isometric absolute strength of *Tibialis anterior (TA)* muscle

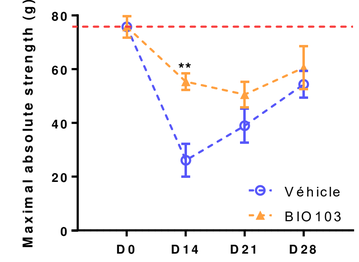


Figure 4: 14 days after hindlimb immobilization, maximal absolute isometric strength (P0) is significantly decreased in vehicle-treated mice compared to non-immobilized control mice (red line) (-65.6%, p<0.001). To a lesser extent, absolute strength is decreased in BIO103-treated mice (-26,9%, p=0,015) compared to control mice. Very interestingly, BIO103-treated muscle develop a stronger strength compared to vehicle-treated mice. BIO103 treatment protects against loss in absolute strength (+112,1%; p=0.004).

→ BIO103 prevents the loss of maximal absolute strength after 14 days of hindlimb immobilization

- Maximal isometric specific strength of *Tibialis anterior (TA)* muscle

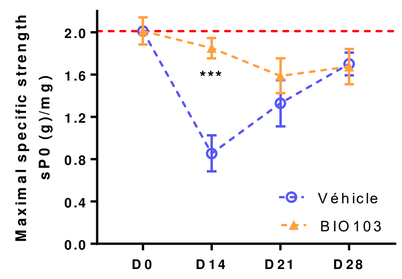
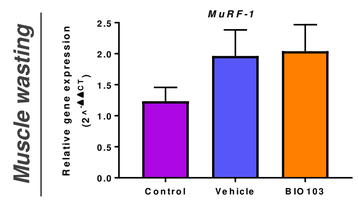


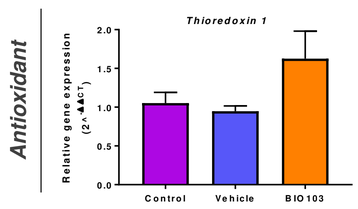
Figure 5: 14 days after hindlimb immobilization, maximal specific isometric strength (sP0) is significantly decreased in vehicle-treated mice compared to non-immobilized control mice (red line) (-57.8%, p<0.001). Importantly, specific strength is not significantly decreased when mice are treated with BIO103 (-8%, p=0.32) compared to control mice. BIO103-treated muscle develop a stronger strength compared to vehicle treated muscles. BIO103 treatment protects against loss in specific strength (+117.6%; p<0.001).

→ BIO103 prevents loss of muscle functionality after 14 days of hindlimb immobilization by preventing the loss of isometric specific strength.

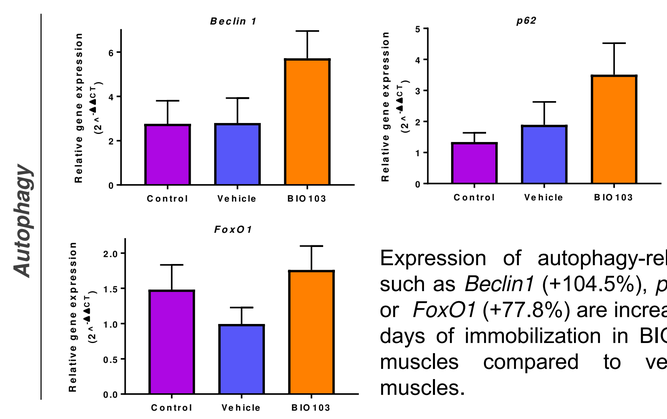
▪ Molecular analysis



Molecular marker for atrophy, Muscle ring finger 1 (*MuRF-1*) mRNA is upregulated 14 days after immobilization in vehicle- and BIO103-treated mice by 1.6- and 1.7-fold, respectively. There is no difference between vehicle and BIO103 treatment.



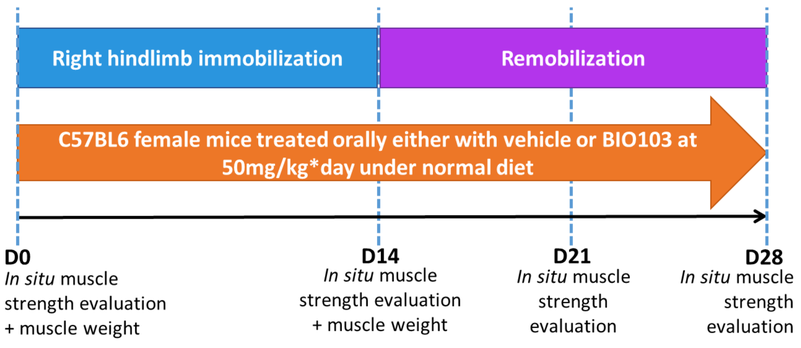
Expression of antioxidant gene *Thioredoxin 1* is increased after 14 days of immobilization in BIO103-treated muscles compared to vehicle treated muscles (+72.3%).



Expression of autophagy-related genes such as *Beclin1* (+104.5%), *p62* (+85.2%) or *FoxO1* (+77.8%) are increased after 14 days of immobilization in BIO103-treated muscles compared to vehicle-treated muscles.

→ BIO103 treatment increases the expression of antioxidant-related genes as well as autophagy-related genes during the immobilization phase

Experimental design



Conclusions

These results demonstrate the efficacy of BIO103 in the preservation of muscle functionality during hindlimb immobilization.

BIO103 significantly increased the absolute strength of 14-day immobilized C57Bl6 mice and increased the expression of antioxidant-related genes which could limit (i) the enhanced oxidative damage during the late phase of disuse muscle atrophy, and (ii) the autophagy-related genes that are able to prevent the accumulation of damaged organelles and maintain cellular homeostasis.

BIO103 could offer a new option for preventing muscle disuse atrophy and loss of strength during immobilization period, which is commonly associated with severe acute and chronic complications in patients.