BIO101, a drug candidate targeting Mas Receptor for the treatment of age-related muscle degeneration. From molecular target identification to clinical development

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Abstract

Background: Above 40, skeletal muscles undergo progressive loss of volume (sarcopenia) and strength (dynapenia). Reduced performances impair mobility and engage a vicious cycle where reduced physical activity results in a further muscle degradation enhanced in obese people due to the deleterious effects of muscle infiltration by adipose tissue (sarcopenic obesity).
Muscle decay results from both reduced protein synthesis and enhanced protein degradation, thus attempts to treat sarcopenia target the muscle cells in order to improve the balance between protein synthesis and degradation processes.
Methods: BIO101 is a pharmaceutical grade preparation of 20-hydroxyecdysone extracted from *Stemmacantha carthamoides*. This molecule was initially investigated *in vitro* on a mouse myocytes cell line (C2C12). Markers of protein synthesis and degradation as well myotubes diameter have been determined to evaluate the effects of BIO101. The nature of BIO101 receptor and subsequent signalling pathways were studied by western blot and pull down assays (see poster 4-02 by Serova et al.).
BIO101 has been further tested by chronic oral administration to 12 and 22-month old C56Bl6/J mice under hyperlipidic diet. The whole animal physical performances, the in situ functionality of *tibialis anterior*, and different molecular markers of selected muscles were measured before and after 4-month treatment.
Results: BIO101 dose-dependently reduced the expression of myostatin, stimulated the phosphorylation of S6K and the incorporation of ^{3}H-leucine, and increased the size of myotubes. These effects involve the activation of Mas, the angiotensin (1-7) receptor. Most importantly, we demonstrated that the treatment of old animals by BIO101 can compensate the significant loss of functionality as a consequence of aging.
Conclusions: our *in vitro* and *in vivo* investigations demonstrate the BIO101 potential in improving skeletal muscle quality in ageing mammals, and justify the clinical development of this drug candidate in sarcopenic patients (see poster 5-01 by Dioh et al.).

Introduction

The aging of the muscles is accompanied by a reduction in the size of fibers, as well as by a change in the distribution of the types of fibers. In particular, the loss of muscle results from a reduction in proteosynthesis linked to the reduction in anabolising factors and by an increase in proteolysis. The involvement of the renin-angiotensin system (RAS) in the physiopathological process leading to sarcopenia was considered in various studies.
In particular, the study of the physical capacity of elderly patients treated with certain ACEI (Angiotensin Conversion Enzyme Inhibitor) provided a demonstration, in intervention studies, that in some cases, this treatment could improve the mobility of elderly patients. Biophytis has oriented its work towards the activation of one of the receptors in the system: the Mas receptor.

Drug candidate:

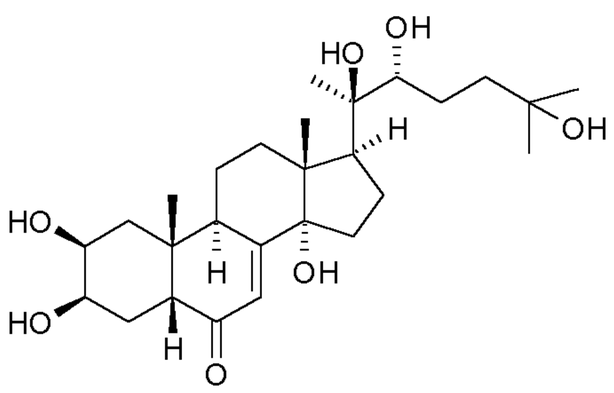

Figure 1. BIO101 is a pharmaceutical grade preparation (>97%, HPLC) of 20-hydroxyecdysone (20E) extracted from *Stemmacantha carthamoides*. 20E belongs the family of ecdysteroids. This molecule is different from mammalian and human steroid hormones and for this reason, does not interfere with their hormone systems.

Results

In vitro studies

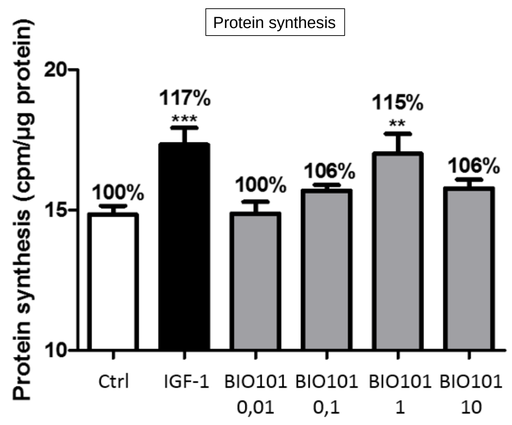

Figure 2: Effect of BIO101 on protein synthesis in C2C12 differentiated cells. IGF1 (100 ng/mL) or BIO101 (0.001-0.1-1-10 µM) in the presence of radiolabeled leucine. Statistical analysis was performed using an Anova followed by a Dunnett t test or a Kruskall Wallis followed by a Dunn's test when the variances significantly differed. *p<0.05, **p<0.01, ***p<0.001 versus control cells

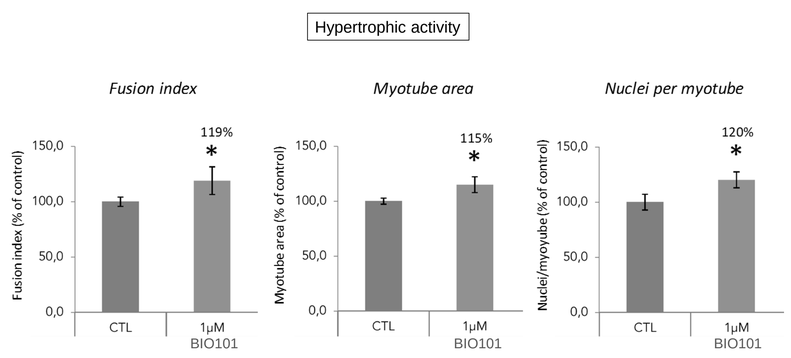

Figure 3: Hypertrophic effects of BIO101 on human myoblasts. Human myoblasts were induced for 6 days into differentiation. After 1 day, BIO101 (1µM) or vehicle were added for 4 days. Cells were fixed then stained for troponin T and nuclei. Images were acquired and analyzed with an Operetta High Content Imaging System from Perkin Elmer. Fusion index: percentage of nuclei located inside myotubes (good indicator of myotube differentiation). Myotube area: proportional to protein synthesis. Nuclei per myotube: indicate the number of myoblasts that fused into a myotube; reflects hypertrophy. Statistical analysis was performed using a Mann Whitney test. *p<0.05

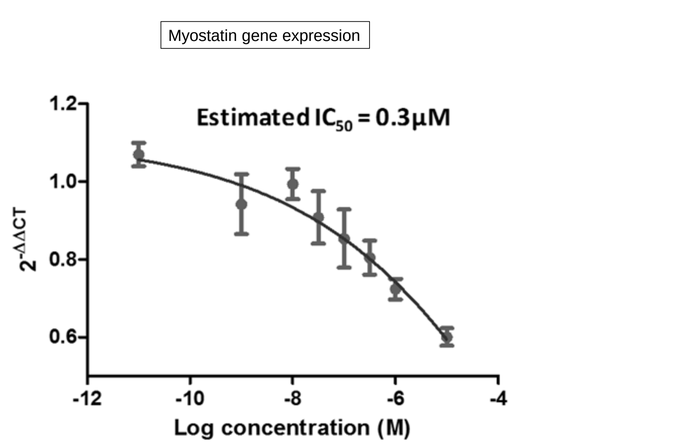

Figure 4: Dose-response curve of myostatin gene expression. Differentiated C2C12 cells were treated with increased concentration of BIO101 (from 0.001µM to 10µM) for 6h. RNAs were extracted, purified and converted into cDNA to allow myostatin gene expression analysis by semi-quantitative PCR. House keeping gene used was beta-actin.

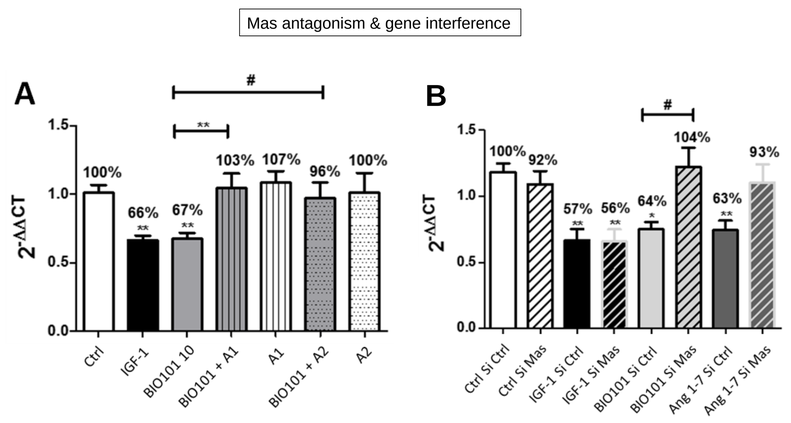

Figure 5. Effects of Mas antagonists and Mas gene interference on BIO101-mediated inhibition of myostatin gene expression. A/ Differentiated C2C12 cells were treated with IGF-1 100ng/mL or BIO101 (10 µM) in the presence or in the absence of Mas antagonists (A1 10 µM or A2 10 µM) for 6 h. B/ Differentiated C2C12 cells were transfected with SiRNA directed against a scramble sequence or against Mas receptor sequence. 2 days after transfection, C2C12 cells were treated with 100 ng/mL IGF-1 or BIO101 (10 µM) or angiotensin 1-7 (Ang 1-7 10 µM) for 6 h. RNAs were extracted, purified and converted into cDNA to allow myostain gene expression analysis by semi-quantitative PCR. Statistical analysis was performed using an Anova followed by a Dunnett t test or a Kruskall Wallis followed by a Dunn's test when the variances significantly differed. *p<0.05, **p<0.01, ***p<0.001 vs control. To compare only two groups a Mann-Whitney statistical test was performed. # p<0.05.

Animal studies

Young animals

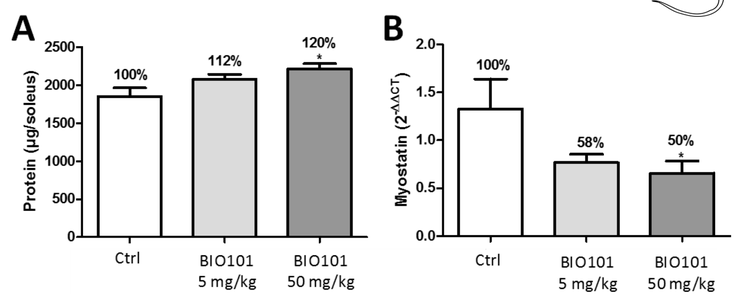

Figure 6: Effects of BIO101 treatment on total protein and myostatin gene expression in soleus muscle. A/Total proteins from soleus muscle (n=10) were measured by Lowry method and normalized to control group (vehicle). B/ Soleus muscle RNAs were extracted, purified and converted into cDNA to allow myostain gene expression analysis by semi-quantitative PCR (n=10). Statistical analysis was performed using an Anova followed by a Dunnett t test. *P<0.05.

Adult vs old animals

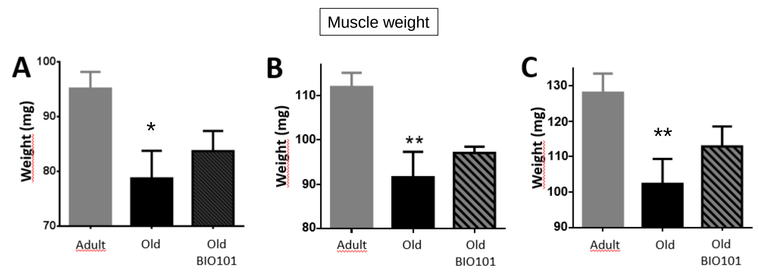

Figure 7: Effects of BIO101 chronic treatment on muscle weight in adult and old mice. Adult (12 months; n=9) and old (22 months) C57Bl6/J mice were orally treated for 14 weeks with either vehicle (n=6) or 50 mg/kg/day of BIO101 (n=7). All animals were submitted to high fat diet. Muscles were isolated and weighed. A/ gastrocnemius muscle (immobilized leg for 7 days). Active leg: gastrocnemius (B) and quadriceps (C) muscles. Statistical analysis was performed using Mann-Whitney test between Adult and Old groups and between Old and Old treated groups *: *P* <0.05 vs adult; **: *P* <0.01 vs adult.

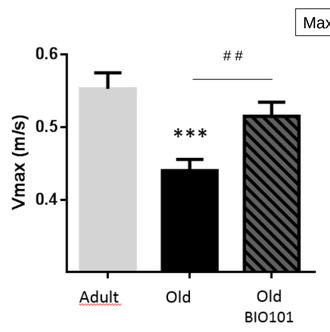

Figure 8: Effects of BIO101 chronic oral treatment on maximal speed in adult and old mice. Adult (12 months) and old (22 months) C57Bl6/J mice were orally treated for 14 weeks with either vehicle or 50 mg/kg/day of BIO101. All animals were submitted to high fat diet. Maximal speed were recorded on treadmill. Statistical analysis was performed using a Mann-Whitney test. ##p<0.01 vs Old. ***p<0.001 vs Adult.

→ Consistently with our in vitro data, chronic oral administration of BIO101 was responsible for a significant increase in animal performance when compared to old untreated animals. Most importantly, we demonstrated that the treatment of old animals by BIO101 can compensate for the significant loss of running velocity as a consequence of aging.

Pharmaceutical and clinical developments

Biophytis has performed both GLP and non-GLP toxicology studies covering in vitro and in vivo genotoxicity, cardiac potassium channel integrity, plasma protein binding, intestinal cell transport, as well as PK and animal toxicological studies in rodents and non-rodent species following acute and chronic administration of BIO101. Results proved satisfactory and no observed adverse effect level (NOAEL) were established in various studies.
SARA-PK is a randomized, double-blind clinical trial in healthy young and older volunteers, evaluating single and multiple ascending oral doses of BIO101. SARA-PK objectives were to evaluate the safety, pharmacokinetics, and food effect of BIO101 administered by oral route (See poster 5-01 by Dioh *et al*.).
SARA-PK results will allow to define the oral doses of SARA-INT, the interventional Phase2 trial to evaluate the efficacy and safety of Sarconeos (BIO101) for the treatment of sarcopenia, including sarcopenic obesity.

Conclusion

BIO101 is a drug candidate for the treatment of sarcopenia with a novel mechanism of action. It is currently tested in clinical trial in healthy young and elderly volunteers.