BIO101, a drug candidate targeting Mas Receptor for the treatment of age-related muscle degeneration.

From molecular target identification to clinical development

Pierre Dilda1, Anne-Sophie Foucault1, Maria Servoa1, Sissi On1, Sophie Rayna1, Stanislas Veillet1, Waly Dih1, René Lafont1,2

1 Biophytis, Parc Biotech, 102 avenue Gaston Roussel, 93230 Romainville, France
2 Sorbonne Universités, UPMC Univ Paris 06, CNRS - Institut de Biologie Paris Seine (BIOSIP), 75005 Paris, France

Abstract

Background: Above 40, skeletal muscles undergo progressive loss of volume (sarcopenia) and strength (dynamypenia). 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 (sarcopenia obesitas). 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 pharmacological grade preparation of 20-hydroxyecdysone extracted from Stemmamachna carthamoides. This molecule was initially investigated in vitro on a mouse myocytes cell line (C2C12). Markers of protein synthesis and degradation as well myocytes 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 Servoa et al.). BIO101 has been further tested by chronic oral administration to 12 and 20-month old C56BL/6J mice under hyperlipic diet. The whole animal performances, the in situ functionality of ibalts 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 p38 and the incorporation of 3H-leucine, and increased the size of myocytes. These effects evolve in 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.

Concluding our in vitro and in vivo investigations demonstrate the BIO101 potential in improving skeletal muscle quality in aging mammals, and justify the clinical development of this drug candidate in sarcopenic patients (see poster 5.01 by Dih 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 Inhibitors) 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.

Drugs candidate:

BIO101 is a pharmaceutical grade preparation (0.7%, HPLC of 20-hydroxyecdysone (20E)) free hydrolysate obtained from Stemmamachna carthamoides. 20E is the main active component of the hydrolysate. This molecule is different from mammalian and human steroid hormones and for this reason, does not interfere with their receptors systems.

Results

In vitro studies

Figure 2: Effect of BIO101 on protein synthesis in C2C12-differentiated cells. GFP (100 ng/ml) was transfected into C2C12 cells at differentiation. After 1 day, BIO101 or DMSO (control) were added for 4 days. Cells were fixed, stained for myosin (red) and nuclei (blue), images were acquired and analyzed with an Operetta High Content Imaging System from Perkin Elmer. Fusion index, percentage of nuclei located inside myotubes, and myotube diameter of myotubes differentiated with and without BIO101 were measured to evaluate protein synthesis. These parameters indicate that BIO101 stimulates myotube formation and enhances myotube size/functionality at the protein level. Statistical analysis was performed using a Mann-Whitney test (p < 0.05).

Figure 3: Hypothetical effects of BIO101 on human myocytes. Human myocytes were induced for 5 days in vitro differentiation. After 1 day, BIO101 or DMSO vehicle were added for 4 days. Cells were fixed stained for myosin (red) and nuclei (blue), images were acquired and analyzed with an Operetta High Content Imaging System from Perkin Elmer. Fusion index, percentage of nuclei located inside myotubes, and myotube diameter of myotubes differentiated with and without BIO101 were measured to evaluate protein synthesis. These parameters indicate that BIO101 stimulates myotube formation and enhances myotube size/functionality at the protein level. Statistical analysis was performed using a Mann-Whitney test (p < 0.05).

Figure 4: Dose-response curve of myotube gene expression. Differentiated C2C12 cells were treated with increased concentration of BIO101 (from 0.0125 to 10 µM) for 4 days. RNAs were extracted, purified and converted into cDNA to allow myotube gene expression analysis by semi-quantitative PCR. Muscle myogenic gene was used as scalar.

Figure 5: SARA101 is a drug candidate targeting Mas Receptor for the treatment of sarcopenia, including sarcopenia obesitas.

Figure 6: Effect of BIO101 on protein synthesis in C2C12-differentiated cells. GFP (100 ng/ml) was transfected into C2C12 cells at differentiation. After 1 day, BIO101 or DMSO (control) were added for 4 days. Cells were fixed, stained for myosin (red) and nuclei (blue), images were acquired and analyzed with an Operetta High Content Imaging System from Perkin Elmer. Fusion index, percentage of nuclei located inside myotubes, and myotube diameter of myotubes differentiated with and without BIO101 were measured to evaluate protein synthesis. These parameters indicate that BIO101 stimulates myotube formation and enhances myotube size/functionality at the protein level. Statistical analysis was performed using a Mann-Whitney test (p < 0.05).

Figure 7: Hypothetical effects of BIO101 on human myocytes. Human myocytes were induced for 5 days in vitro differentiation. After 1 day, BIO101 or DMSO vehicle were added for 4 days. Cells were fixed stained for myosin (red) and nuclei (blue), images were acquired and analyzed with an Operetta High Content Imaging System from Perkin Elmer. Fusion index, percentage of nuclei located inside myotubes, and myotube diameter of myotubes differentiated with and without BIO101 were measured to evaluate protein synthesis. These parameters indicate that BIO101 stimulates myotube formation and enhances myotube size/functionality at the protein level. Statistical analysis was performed using a Mann-Whitney test (p < 0.05).

Figure 8: Dose-response curve of myotube gene expression. Differentiated C2C12 cells were treated with increased concentration of BIO101 (from 0.0125 to 10 µM) for 4 days. RNAs were extracted, purified and converted into cDNA to allow myotube gene expression analysis by semi-quantitative PCR. Muscle myogenic gene was used as scalar.

Figure 9: SARA101 is a drug candidate targeting Mas Receptor for the treatment of sarcopenia, including sarcopenia obesitas.

An old adult animal with BIO101 performed a better performance in the treadmill test than the group of old control animals.

Pharmacological 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 toxicology studies in rodents and non-rodent species following acute and chronic administration of BIO101. Results proved satisfactory and no adverse effect level (NOAEL) was established in various studies. SARA-PK is a randomized, double-blind clinical trial in healthy young and elderly volunteers, evaluating single and multiple ascending oral doses of BIO101. SARA-PK objectives were to evaluate the safety, pharmokinetics, and food effect of BIO101 administered by oral route (see poster 8.01 by Dih et al.).

SARA-PK results will define the oral doses of SARA-INT, the Interventions-Phases trial to evaluate the efficacy and safety of Sarcoines (BIO101) for the treatment of sarcopenia, including sarcopenia obesitas.

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.