Heat shock hits muscular dystrophy

By Tim Fulmer, Senior Writer

Australian academics have used N-Gene Research Laboratories Inc.'s BGP-15, a small molecule inducer of heat shock 70 kDa protein 1A in Phase IIb testing for diabetes, to increase muscle strength and survival in two mouse models of muscular dystrophy.¹ The biotech now hopes to find a partner to help repurpose the compound for muscular dystrophy.

The lack of a functioning dystrophin (DMD) protein is the underlying cause of muscular dystrophy, but chronic muscle inflammation and loss of intracellular calcium homeostasis are what actually drive disease progression. Multiple proinflammatory signaling molecules, including tumor necrosis factor- α (TNF- α), NF- κ B and c-jun N-terminal kinase (JNK), are upregulated in the muscle tissue of animal models of muscular dystrophy.²⁻⁴

Because there are so many inflammatory factors at work in the disease, the challenge has been identifying a single target that inhibits the activity of multiple pathways in muscle.

The Australian researchers hypothesized that upregulating the heat shock response could accomplish the task. The team focused on heat shock 70 kDa protein 1A (Hsp72; HspA1), which inhibits NF- κ B and JNK signaling and preserves the function of the calcium pumps ATPase Ca⁺⁺ transporting cardiac muscle fast twitch 1 (ATP2A1; SERCA1) and ATPase Ca⁺⁺ transporting cardiac muscle slow twitch 2 (ATP2A2; SERCA2A).⁵⁻⁸

The team crossed muscle-specific Hsp72-overexpressing mice with *mdx* mice, which lack the *dystrophin* gene and are the standard muscular dystrophy mouse model.

The resulting mice had significantly decreased whole-body muscle breakdown ($p<0.05$), increased strength and endurance ($p=0.002$) and better diaphragm histology ($p<0.05$) compared with *mdx* controls not overexpressing Hsp72.

In quadriceps muscles from the crossbred mice, the function of all SERCA pumps was increased compared with that in *mdx* mice ($p<0.008$), suggesting that Hsp72 overexpression protected SERCA from inactivation and helped preserve calcium homeostasis in muscle cells.

The researchers next asked whether they would see a similar result delivering an Hsp72-inducing small molecule to the *mdx* mice. They

turned to N-Gene's BGP-15, which is in a Phase IIb trial to treat type 2 diabetes.

In that disease, the induction of Hsp72 blocks activation of JNK-associated proinflammatory pathways that contribute to insulin resistance. In its Phase I and Phase II diabetes trials, BGP-15 was safe and well tolerated.^{9,10}

In the *mdx* mice, BGP-15 decreased both whole-body muscle breakdown ($p<0.01$) and diaphragm fibrosis ($p<0.05$) and increased both diaphragm strength ($p<0.01$) and SERCA activity in diaphragm muscle ($p<0.05$) compared with vehicle.

Finally, the researchers delivered BGP-15 to dystrophic *dko* mice, which have double knockout of the *utrophin* and *dystrophin* genes and a much more severe disease phenotype than *mdx* mice. In the *dko* animals, BGP-15 decreased spinal curvature ($p<0.05$) and increased both muscle function ($p<0.01$) and lifespan ($p<0.05$) compared with vehicle.

Results were published in *Nature*. The team was led by Gordon

Lynch, head of the Department of Physiology at The University of Melbourne, and Mark Febbraio, senior principal research fellow and head of the Cellular and Molecular Metabolism Laboratory at the Baker IDI Heart & Diabetes Institute.

Based on the *dko* mouse data, "it is likely that BGP-15 will work at least as well if not better than the other pharmacological approaches currently used in DMD [Duchenne muscular dystrophy] patients," such as corticosteroids, said Kay Davies, professor of physiology, anatomy and genetics at the University of Oxford.

Davies provided the *dko* mice used in the *Nature* study.

Activating Hsp72 in muscular dystrophy "has the potential to delay when patients will lose the ability to walk, delay when they'll need machines to help them breathe and potentially allow them to live longer with a better quality of life," Lynch told SciBX.

Right patients, more indications

Eventually, Lynch, Febbraio and N-Gene plan to combine resources to run a trial of BGP-15 in muscular dystrophy. They also hope to find a partner to help them with the clinical development. They declined to provide a more detailed timeline.

Lynch said that prior to any clinical trials of BGP-15, additional work in the dystrophic mice should help determine the profiles of patients who might benefit most from BGP-15. In particular, the researchers want to know whether treatment must start before a certain stage of disease to be effective and whether there is a specific treatment window to produce beneficial effects on muscle structure and function.

The findings showed that BGP-15 slowed disease progression when the animals were treated from as early an age as possible, said Lynch, "which is similar to a scenario where boys with DMD are placed on corticosteroids as soon as possible after diagnosis to have the best effect

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—Gabor Kalman,
N-Gene Research Laboratories Inc.

at slowing progression.”

“We now want to establish in the mice whether starting treatment at a later time point will provide a benefit, especially if there is a significant infiltration of fibrosis and other noncontractile material,” he continued.

“At this time we are not in a position to develop BGP-15 for diabetes and muscular dystrophy in parallel,” N-Gene CEO Gabor Kalman told *SciBX*. “But at some point in the future, depending on the outcome of the ongoing diabetes trial, we would very much like to pursue this compound in muscular dystrophy, and we want to find a partner to help us move forward.”

Hsp72 inducers like BGP-15 may have therapeutic effects in a broad range of diseases associated with stress-induced molecular damage and protein unfolding. “When activated by cellular stress, Hsp72 is a chaperone protein that prevents a misfolded protein response in the cell,” thus preserving the function of key proteins and helping to better maintain cellular homeostasis, said Febbraio. “BGP-15 could be useful in other diseases where a misfolded protein response is prevalent,” such as Alzheimer’s disease (AD).

BGP-15 “also may be efficacious for the treatment and management of numerous muscle diseases as well as nonmuscle diseases that present with skeletal muscle weakness and dysfunction,” said Russell Tupling. The reason, he said, is the compound “maintains normal SERCA pump function, allowing the damaged muscle cells to better deal with calcium influx, minimizing calcium overload and associated muscle damage and degeneration.”

Tupling is professor of kinesiology at the **University of Waterloo**. Tupling and colleagues previously showed that Hsp72 protects SERCA, thus preventing oxidative damage and cellular dysfunction.^{8,11}

N-Gene has a series of small molecule heat shock protein inducers in preclinical development behind BGP-15. Early in the development of

BGP-15 and its amidoxime analogs, N-Gene researchers and colleagues showed that the compound class had cytoprotective effects in diabetic wound healing, cardiac ischemia and cardiac reperfusion injury.^{12,13}

N-Gene, Lynch and Febbraio have applied for a patent covering the use of BGP-15 to treat muscular dystrophy and other muscle disorders, Kalman said. Febbraio is a scientific consultant for N-Gene.

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