

Identification Of Compounds Enhancing Utrophin Expression In Primary Human Skeletal Muscle Cells

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Introduction

Duchenne Muscular Dystrophy (DMD)

DMD is the most prevalent, genetically inherited neuromuscular disorder worldwide and affects 1 in 3500 young males. This disorder is caused by mutations or deletions in the gene encoding dystrophin that prevent the synthesis of full-length dystrophin molecules in skeletal muscle fibres. The lack of dystrophin in muscles of DMD patients leads to sarcolemmal instability and disruption of the dystrophin-associated protein complex (DAPC). Together with dystrophin, DAPC forms a link between the extracellular matrix and the intracellular actin cytoskeleton, thereby providing structural integrity to muscle fibers. In DMD patients, the regenerative potential of dystrophic muscle fibers diminishes over time, resulting in progressively severe muscle necrosis and wasting. This loss of muscle mass causes patients to be confined to wheelchairs in their early teenage years and to die by the second or third decade of life as a result of cardiac or respiratory failure. Although the molecular defect responsible for DMD was identified 20 years ago, there is still no effective treatment available for this devastating disease. Doctors recommend daily stretching and the use of corticosteroids that provides minimal short term benefits.

Aim

A promising pharmacological treatment for DMD aims to increase levels of utrophin, a fetal homologue of dystrophin, in muscle fibers of affected patients to compensate for the absence of dystrophin. Studies have indicated that expression of utrophin in adult murine muscle cells in mouse models for dystrophy prevents dystrophy pathology. Thus, induction of utrophin in muscle cells may be of therapeutic value in DMD patients. To this aim, BioFocus DPI developed a cell-based assay in which the effect of chemical compounds on utrophin up-regulation could be quantified. The assay was subsequently used to screen the NIH Clinical Collection (NCC), a plated array of 446 small molecules that have a history of use in phase I-III of human clinical trials.

Materials and Methods

Cells

Normal human muscle myoblasts (H5MM) originating from a 16 years old caucasian male were obtained from Lonza Verviers SPRL (Belgium). After two passages, fifty percent confluent myoblasts were switched from culturing medium to differentiation medium (DMEMF-12 supplemented with 2% horse serum) that promoted myoblasts fusion into multinucleated myotubes over 5 days. Differentiation was confirmed by myosin immunostaining and cells morphological assessment using titin immunostaining combined with DAPI nuclear staining.

Utrophin quantification assay

BioFocus DPI has successfully developed a cell-based assay measuring utrophin up-regulation by human primary myotubes in response to 2 different stimuli: a recombinant growth factor, heregulin or a specific phosphatase inhibitor, okadaic acid. Briefly, myotubes were stimulated over myoblast differentiation with a concentration range of the stimulating agent. Cells were lysed and utrophin was subsequently measured in the lysates on the Mesoscale Discovery (MSD) platform. The MSD assay was optimized as a sandwich immunoassay using utrophin present in lysates of the murine skeletal muscle cell line Sol8. In the assay, utrophin was specifically captured by a polyclonal antibody and detected by a murine monoclonal antibody in combination with an anti-mouse labelled with MSD SULFO-TAG™ label.

Screen of the NIH Clinical Collection

The NIH Clinical Collection (NCC) contains 446 small molecules that have been tested in human clinical trials and have highly developed properties of drug-likeness. Each compound was tested in the assay described above in biological duplicate at two concentrations: 10 and 5 µM in differentiation medium. A dilution range of heregulin (500, 100 and 10 nM) and of okadaic acid (7.5, 5 and 2.5 nM) selected as positive controls were included on each assay plate with the appropriate vehicle.

Results

Utrophin quantification assay

Assay readout optimization

