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Session 3: How reliable are animal models?

The mdx mouse for pre-clinical studies of new therapeutics for Duchenne Muscular Dystrophy
Animal models in Duchenne Muscular Dystrophy

Golden retriever muscular dystrophy dog (GRMD)

Mdx mouse

Pros

- Genetically and biochemically homolog of DMD
- Presence of some typical markers of disease
- Practical to be used for studying pathogenesis and for testing drugs and gene-based approaches

Pathogenetic mechanisms and drug targets

Absence of dystrophin

Dislocation of nNOS

Contraction-induced functional ischemia

Defective DGC signaling and metabolic adaptation

Mechanical activation of NADPH ox

ROS

Fatigue and metabolic failure

Inflammation and oxidative stress

Pro-inflammatory cytokines (TNF-α, IL-1, etc.)

Pro-fibrotic cytokines (TGF β, Myostatin)

ROS-induced membrane peroxidation

Myofiber death, regeneration failure and fibrosis

Activation of proteases

Calcium overload

Altered calcium homeostasis

Activation of calcium channels mechanosensitive and/or leak, SR alteration, SOCE
Is mdx a good DMD model in preclinical tests?

**EMA DRAFT**

**7.2. Pharmacodynamics**

The proposed mechanism of action of a new product should be described and discussed in relation to possible testing in available animal models which are currently limited. (e.g. the mdx mouse is considered a poor model of the DMD phenotype, while the predictive value of results in the golden retriever muscular dystrophy dog is still unknown)....

**Bottlenecks**

- Mild phenotype
- Variability in experimental approaches
- Difficulties in defining hard and surrogate endpoints
- Editorial bias (few negative or confirmatory data)
- Failure of some promising candidate drugs in clinical settings
Task-force for translational research in DMD

- Harmonize experimental studies in animal models
- Provide freely-accessible Standard Operating Procedures
- Suggest guidelines

17 SOPs on mdx mice
4 SOPs on GRMD dogs
5 Reviews / articles

http://www.treat-nmd.eu/research/preclinical/SOPs
Enhancing translation: Guidelines for standard pre-clinical experiments in mdx mice.


“Proof of concept” versus “pre-clinical therapeutic” studies

Early phase versus later phase experiments

Methodological Standards

1. Animal care: age, gender, housandry, etc

2. Experimental design

   2.1. Outcome measure selection

      2.1.1. Core set of parameters in mdx pre-clinical efficacy studies

      2.1.2. Optional set of parameters in mdx pre-clinical efficacy studies

   2.2. Muscle selection

   2.3 Sample Size and Power

   2.4 Randomization and Blinding

   2.5 Exercise

   2.6 Age at treatment start and at terminal sampling

3. Reporting Results

   3.1 The Recovery Score

   3.2 Anticipated translational benefit
Guidelines for pre-clinical drug tests in animal models

Perspective

A call for transparent reporting to optimize the predictive value of preclinical research


doi:10.1038/nature11556

The US National Institute of Neurological Disorders and Stroke convened major stakeholders in June 2012 to discuss how to improve the methodological reporting of animal studies in grant applications and publications. The main workshop recommendation is that at a minimum studies should report on sample size estimation, whether and how animals were randomized, whether investigators were blind to the treatment, and the handling of data. We recognize that achieving a meaningful improvement in the quality of reporting will require a concerted effort by investigators, reviewers, funding agencies and journal editors. Requiring better reporting of animal studies will raise awareness of the importance of rigorous study design to accelerate scientific progress.
One strategy: the exercised mdx mouse

AIM
to aggravate the murine phenotype based on the mechanical and growth susceptibility of dystrophin-deficient muscles.
In vivo “patients-like” outcome measures

http://www.treat-nmd.eu/research/preclinical/SOPs

Exercise leads to:

significant decrease in mouse force

*De Luca et al., JPET 2003; Neurobiol Dis., 2008*

worsening of fatigue and function (in endurance tests)

*Burdi et al., J Appl Physiol., 2009; Cozzoli et al., Pharmacol Res 2012*
In vivo force and drugs

Time and intensity-related effects

Some drugs act sooner....

GLPG0492, a novel selective androgen receptor modulator, improves muscle performance in the exercised-mdx mouse model of muscular dystrophy.

Anna Cozzoli\textsuperscript{1,2}, Roberta Francesca Capogrosso\textsuperscript{2,3}, Valeriana Teresa Stiberdorio\textsuperscript{4}, Maria Maddalena Einarde\textsuperscript{4}, Catherine Jagerchmidt\textsuperscript{4}, Florence Nantour\textsuperscript{5}, Giulia Maria Camerino\textsuperscript{6}, Annamaria De Luca\textsuperscript{4,6}

...others require longer

Gentamicin treatment in exercised mdx mice: Identification of dystrophin-sensitive pathways and evaluation of efficacy in work-loaded dystrophic muscle.

Annamaria De Luca\textsuperscript{4,6}, Beatrice Nico\textsuperscript{7}, Jean-François Rolland\textsuperscript{7}, Anna Cozzoli\textsuperscript{7}, Rosalba Berti\textsuperscript{8}, Domenico Margerin\textsuperscript{9}, Viviana Giannari\textsuperscript{7}, Antonella Lianos\textsuperscript{8}, Valentina Cipresso\textsuperscript{8}, Michela De Bellis\textsuperscript{9}, Grazia Paula Nocchi\textsuperscript{8}, Giulia Maria Camerino\textsuperscript{7}, Antonio Frigeri\textsuperscript{7}, Maria Stefello\textsuperscript{7}, Dana Conte Camerino\textsuperscript{6}

\textsuperscript{1}Neurobiology of Disease 32 (2008) 243–253
In vivo fatigue and drugs

Dystrophins carrying spectrin-like repeats 16 and 17 anchor nNOS to the sarcolemma and enhance exercise performance in a mouse model of muscular dystrophy

Yi Lui,1 Garth D. Thomas,2 Yongping Yue,3 Helios T. Yang,3 Dejia Li,3 Chun Long,3 Luke Judge,4 Brian Bodick,4 Jeffery S. Chamberlain,4 Ronald L. Terjung,3 and Dongsheng Duan*3

The Journal of Clinical Investigation March 2009

Reinforcement of sarcolemma or stimulation of anabolism

Enhancement of NO and related signalling

NO-flurbiprofen
Brunelli et al., PNAS 2007

Pentoxifylline
Burdì et al., J Appl Physiol. 2009

Modified from Verhaert et al.
Ex vivo / In situ hard endpoints

Histopathology

Muscle Physiology

Weakness

Susceptibility to eccentric contraction

Dystrophin/utrophin expression

Willmann et al., NMD, 2012
Conclusions

• The mdx mouse is a valuable model for studying new therapeutics for DMD, if properly used;

• A great effort has been done by the wide scientific community to create, upgrade and divulgate Standard Operating Procedures and guidelines with improved predictability of pre-clinical data;

• Translational research is enhanced by increased communication between pre-clinical scientists, clinicians, regulatory agencies, patient’s association and drug discovery companies.