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Enhancing translation: Guidelines for standard pre-clinical experiments in *mdx* mice

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Abstract

Duchenne Muscular Dystrophy is an X-linked disorder that affects boys and leads to muscle wasting and death due to cardiac involvement and respiratory complications. The cause is the absence of dystrophin, a large structural protein indispensable for muscle cell function and viability. The *mdx* mouse has become the standard animal model for pre-clinical evaluation of potential therapeutic treatments. Recent years have seen a rapid increase in the number of experimental compounds being evaluated in the *mdx* mouse. There is, however, much variability in the design of these pre-clinical experimental studies. This has made it difficult to interpret and compare published data from different laboratories and to evaluate the potential of a treatment for application to patients. The authors therefore propose the introduction of a standard study design for the *mdx* mouse model. Several aspects, including animal care, sampling times and choice of tissues, as well as recommended endpoints and methodologies are addressed and, for each aspect, a standard procedure is proposed. Testing of all new molecules/drugs using a widely accepted and agreed upon standard experimental protocol would greatly improve the power of pre-clinical experimentations and help identifying promising therapies for the translation into clinical trials for boys with Duchenne Muscular Dystrophy.

Keywords: DMD; *mdx*; Methods; Standard operating procedures; Pre-clinical experiments

Introduction

The last few years have witnessed increasing efforts to evaluate potential therapeutic compounds for Duchenne Muscular Dystrophy (DMD) in the *mdx* mouse model. A plethora of data was published reporting important effects of various strategies, making it difficult to select the best candidates for human studies and often facing less enthusiastic or virtually no positive results in patients. The risk of false positive or false negative is still very high, due to intrinsic problem in animal models as well as improper definition of either study-design or readout parameters. Indeed, these can vary considerably with respect to important methodological features such as the selection of the outcome measures and the choice of sampling times for assessment (see Table 1 in [1] and in [2]), confounding comparison of results and hindering the prioritization of human clinical trials in already rare and restricted patient

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populations. One way to avoid this is to do animal experiments in the same manner as human clinical trials, using a standardized and methodologically rigorous approach to pre-clinical experimental studies in *mdx* [3–5], similar to those published recently for pre-clinical trials in ALS/MND and stroke [6–8].

The formulation of these recommendations was initiated by a workshop held in Brazil in 2006 [9] and continued in two workshops held in 2007 and 2008 [10]. In an effort to attain a consensus on the methods used in mouse pre-clinical studies, a group of scientists has come together under the auspices of the multi-national TREAT-NMD network (http://www.treat-nmd.eu/). This manuscript summarizes the deliberations of this group with respect to standardization of methods and proposes a number of procedures that relatively easily could be implemented in pre-clinical therapeutic studies.

"Proof of concept" versus "pre-clinical therapeutic" studies

At the outset, we wish to make a distinction between what has been termed "proof-of-concept" and "pre-clinical therapeutic" studies. The former are early stage exploratory studies that raise treatment hypotheses, which then require formal evaluation in pre-clinical "therapeutic" studies. The recommendations of this working group regarding experimental standardization should not be construed as an inhibition of scientific creativity in such initial proof-of-concept studies. However, pre-clinical therapeutic studies, meant to demonstrate the efficacy of a potential therapy, need to be designed with appropriate methodological rigor. A standardization of experimental conditions will facilitate comparison of the relative benefits of treatments evaluated by different investigators in different laboratories. The goal is to improve the speed and efficiency of translation from the laboratory to patients in the clinic.

Early phase versus later phase experiments

The progression of pathology in the *mdx* mouse is influenced by growth [11] and may be divided into three main phases: the pre-weaning phase (0–3 weeks of age) which is strongly influenced by growth and corresponds roughly to the first 6 months of human patients (see Box 1 in [4]); the post-weaning phase, with an acute onset of pathology around 3 weeks, followed at about 8 weeks by the adult phase with a reduced low level of chronic damage that persists throughout life. Experiments in the pre-weaning phase aim to evaluate the ability of a drug to reduce/delay the first round of necrosis. These studies on very young mice, which may not practically be available from central breeders, are frequently performed on pups of the same litter, without gender distinctions, from in-house colonies. These should be replenished every 20 generations to avoid gene drifting (see also http://jaxmice.jax.org/geneticheath/drift.html) and care should be taken to optimize litter size and to avoid any unnecessary stress (see below). Also, the number of pups per cage is known to affect post-natal growth due to issues of feeding [12]; this relates to the strength of stimulus for milk production (may be a problem with small litters) and sufficient availability of milk supply (an issue with large litters). Ideally therefore, numbers of pups in litters can be adjusted neonatally by moving pups between mothers to increase or decrease the number and provide a standardised number in each litter (e.g. seven pups/mother).

The guidelines proposed in the following chapters of this manuscript are relevant to standardization of experiments in the post-weaning and adult phase.

Methodological standards

There are a number of methodological issues that require standardization. These include animal care, several aspects of experimental design (including randomization, outcome measure selection, blinding and consideration of sample size and power) as well as strategies for reporting study results.

1. Animal care

A number of aspects of animal care may contribute to the variability observed in mice from different laboratories (see data collection in [2]). A standardized approach to animal care is expected to improve the reproducibility of the mouse dystrophic phenotype between experiments and laboratories. Grounds et al. [4] provided a detailed analysis of the impact of animal care on animal physiology; key measures to avoid such confounders are summarized as follows:

- Purchase mice from central distributors. This also potentially allows for a larger number of age-matched, same gender animals to be available within a reasonable timeframe, shorter than would normally be possible when relying on in-house animal breeding facilities.
- Report food regimen. A comparison of food composition from several food suppliers, including the standard diet used by Jackson Labs for *mdx* mice, showed great differences in the content of vitamins, minerals and crude fat (data not shown). Changing the source of the protein content from soy to casein, or from meat to fish, has dramatic consequences on some biological events and may affect the impact of a drug trial [4]. It is therefore recommended that within the same laboratory, the supplier of mouse diet be carefully maintained constant throughout different drug trials, thus avoiding changes in diet. It is advisable to report the food supplier in published data and manuscripts, and eventually, food composition should be considered when interpreting the results. Cage bedding should also be considered as some bedding (e.g. wheat kernels) contains high levels of vitamin E (anti-oxidant) and can