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How clinical trials of myasthenia gravis can inform pre-clinical drug development

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ABSTRACT

Pre-clinical evaluations often provide the rationale for therapeutic assessments in humans; however, in many diseases an agent found successful in animal models does not show efficacy in human subjects. Our contention is that the approach of rigorous, clinical trials can be used to inform how preclinical assessments should be performed. Clinical trials in humans are carefully designed investigations executed with consideration of critical methodological issues, such as pre-specified entrance criteria and validated, outcome measures coupled with power analysis to identify sample size. Blinding of evaluators of subjective measures and randomization of subjects are also critical aspects of trial performance. Investigative agents are also tested in subjects with active disease, rather than prior to disease induction as in some pre-clinical assessments. Application of standard procedures, including uniform reporting standards, would likely assist in reproducibility of pre-clinical experiments. Adapting methods of clinical trial performance will likely improve the success rate of therapeutics to ultimately achieve human use.

Introduction

The armamentarium of immune suppressive agents used in the treatment of autoimmune myasthenia gravis (MG) has largely emanated from the transplant literature and experience with the use of these drugs in the treatment of other autoimmune disorders (Sieb, 2014). At first glance, this may seem surprising given that most common antigenic targets of the autoimmune response in MG are well known and the availability of a well-studied animal models of MG either experimental autoimmune myasthenia gravis (EAMG) produced by immunization with the acetylcholine receptor (AChR) or muscle specific tyrosine kinase (MuSK) or passive transfer of autoantibody (PTMG) (Baggi et al., 2012; Berrih-Aknin and Le Panse, 2014). With the exception of the C5 complement inhibitor eculizumab (Howard et al., 2013), which was originally found to be effective in PTMG rat (Zhou et al., 2007), none of the therapies currently used for treatment of MG emerged from pre-clinical work in animal models. Upon closer inspection, however, it is clear that there are limitations to EAMG as a tool for pre-clinical assessment of potential therapeutic agents, and these issues will be discussed in greater detail elsewhere in this special issue. Here we focus on how experience from clinical trials in patients with MG might be used to enhance the utility of the EAMG rodent models for pre-clinical evaluation of therapeutics prior to their advancement into human clinical trials.

Trial design

Human clinical trials are carefully designed experiments with significant attention to important methodological issues. For example, eligibility criteria are defined in order to yield an appropriate study population; treatment allocation is randomly assigned; primary and secondary outcome measures and endpoints are pre-specified; outcomes are assessed by an evaluator blinded to treatment assignment; and due consideration is given to the sample size needed to demonstrate the minimal clinically important difference in outcome in order to ensure that the trial has adequate power to detect the treatment effect of interest. The rigor of pre-clinical therapeutic studies in EAMG rodent models would benefit from attention to these methodological issues (Table 1).

Abbreviations: MG, myasthenia gravis; AChR, acetylcholine receptor; MuSK, muscle specific kinase; EAMG, experimentally acquired myasthenia gravis; PTMG, passive transfer myasthenia gravis

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Table 1
Recommendations for preclinical assessment based on human studies.

- Inclusion of males and females
- Power calculations to ensure adequacy of sample size
- Use of appropriate statistical methods; avoid unplanned interim analyses
- Treatment initiation after the appearance of clinical disease
- Assessment of the influence of age on therapeutic response
- Improvement by 2 weakness grades
- Randomized allocation to treatment groups
- Blinded assessments of all outcome measures
- Use of quantitative (i.e. more objective) outcome measures
- Biomarker development for preclinical and clinical trials
- Replication of positive pre-clinical findings in a second (independent) laboratory
- Preclinical model validation using therapies known to be effective in human MG

Eligibility criteria

Because human MG has clinical and pathophysiological heterogeneity that is influenced by age and gender (Berrih-Aknin et al., 2014), clinical trials for MG typically restrict enrollment to individuals in a specified age range, although, generally, they are open to both sexes. Animal investigations typically utilize rodents that are in early adulthood and utilize only one sex. Two year old rats have been found to be resistant to development of weakness produced by active immunization with AChR (Hoedemaekers et al., 1997). Old, female rats demonstrated greater loss of AChR than male counterparts but still did not show weakness compared to young rats. The effects appear to result from the properties of the neuromuscular junction rather than age-related changes of the immune system. To align the preclinical studies to those in MG the National Institutes of Health has recommended that preclinical studies be carried out in animals of both sexes (Clayton and Collins, 2014).

Eligibility criteria for human MG clinical trials typically exclude patients with purely ocular disease as well as those with impending or actual myasthenic crisis, and require that patients have some minimal degree of weakness (e.g. Quantitative MG score of at least 12 points). A similar approach might be adopted in the rodent models, requiring that immunized animals develop some minimal degree of weakness (e.g. grade II). It may be far more difficult, for example, to demonstrate a clinical effect in terms of improved strength, in animals that do not have at least grade II weakness prior to administration of the potential therapeutic agent.

Randomization

The principal goal of randomization is to control for potential confounding factors. Some might argue that the potential for confounding factors is low given that the animals used all have the same genetic background, are all housed in the same animal facilities and are exposed to the same environmental factors, thereby mitigating the need for randomization. Experience from the animal model literature in other diseases, however, provides strong evidence for the presence of confounders (e.g. gender, litter effects, gene copy number in genetic models of disease) (Scott et al., 2008) and supports the contention that treatment allocation should always be randomized (Benatar, 2007).

Blinded assessment of outcome

Clinical trials routinely incorporate procedures to ensure that the evaluator responsible for assessing outcome is blinded to treatment allocation. The absence of blinding, especially for outcome measures that include a subjective component, introduces the potential for bias. While the potential for bias similarly exists in pre-clinical therapeutic studies, much less attention has historically been paid to the importance of blinding. However, it can be argued that investigators and laboratory personnel will have similar potential for having a priori expectations for certain results, and therefore blinded assessments should be performed for any potentially subjective outcome measures.

Sample size, power and reproducibility

Sample size calculation is an essential ingredient to ensure that clinical trials are adequately powered. Estimating sample size is predicated upon specification of the primary outcome measure and determination of the minimal clinically important difference that would be interpreted as evidence of a therapeutic effect. Such considerations are uncommon in pre-clinical therapeutic studies, with rationalizations, such as cost considerations, used to justify underpowered studies. Recognition of the potential for false positive and false negative results in small studies should serve as a rallying cry to ensure that pre-clinical therapeutic studies are adequately powered. Statistical methods should be appropriate to the data being analyzed and caution should be taken to avoid interim analyses that have not been pre-specified. Every effort should be made to publish both positive and negative results so that publication bias does not skew our collective perspective on the potential utility of putative therapies. In the same way that federal regulations require pre-registration of human therapeutic studies (e.g. on clinicaltrials.gov) and journal editors refuse to publish unregistered trials, a similar approach (e.g. preclinicaltrials.gov) might help to minimize the potential for publication bias. Lastly, positive pre-clinical studies should be reproduced by independent investigators, especially given the demonstrated variability of animal experiments among laboratories (Landis et al., 2012).

Timing of therapeutic intervention

Unlike MG in humans, which appears to arise spontaneously, EAMG requires induction either via passive administration of antibody from MG patients or rodents with EAMG, or via active immunization with antigen (AChR or MuSK), usually in combination with adjuvant. Canine MG does mimic human MG in that it arises spontaneously, but its use as a preclinical therapeutic model has been limited by the 90 percent remission rate in untreated dogs (Shelton and Lindstrom, 2001).

Depending on the animal species, EAMG may require single dose (rats) or repeated immunization (mice) (Baggi et al., 2012; Christadoss et al., 2000). In the animal models, therefore, the question arises of when to initiate treatment. Historically, many studies have initiated therapy following immunization but prior to the emergence of clinical disease. This approach more closely reflects disease prevention than treatment of established disease, and does not mirror the clinical context, in which it is not possible to initiate treatment prior to the onset of MG. Therapeutic efficacy in the pre-clinical model, therefore, is perhaps more likely to translate into human efficacy if investigative drugs are administered to animals with EAMG after the appearance of clinical manifestations of disease.

Outcome measures and biomarkers

MG clinical trials have historically relied upon measures of muscle strength and fatigue such as the Quantitative Myasthenia Gravis (QMG) scale (Jaretzki et al., 2000). More recently, however, with the growing awareness of the fluctuating nature of myasthenic manifestations and increasing emphasis on patient reported outcome measures, there has been a shift towards using outcome measures such as the MG Quality of Life-15, the MG-Composite and the MG Activities of Daily Living scales that incorporate, or rely entirely, upon patient self-report (Benatar et al., 2012; Wolfe et al., 2008). Importantly, such outcome measures are not feasible for use in pre-clinical animal therapeutic studies, which instead typically rely upon grades of disease

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