Checklist for reporting and reviewing studies of experimental animal models of multiple sclerosis and related disorders

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ABSTRACT

Animal models of neurodegenerative and inflammatory diseases have greatly contributed to our understanding of human disorders such as multiple sclerosis (MS). These models play a key role in drug development and have led to novel therapeutic approaches to treat human diseases. Nevertheless, some studies showing efficacy of therapies in animal models have not translated well to the clinic. In part, this disparity can be explained by differences in the biology of animals and humans. Another contributing factor is the quality of execution and reporting of studies, which is the responsibility of the authors. However, the acceptance of these papers depends on the quality of refereeing and editorial proficiency. When reporting animal studies, it is recommended that manuscripts conform to the principals of the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines (Kilkenny et al., 2010). This provides a list of 20 guidelines that should be employed in order to make papers consistent as well as transparent. However, conformation to the ARRIVE guidelines requires significantly more information than current publications often report.

We have thus refined the ARRIVE guidelines, incorporated the 3Rs (Reduction, Refinement and Replacement) principals, and specifically adapted them to the reporting of animal models of multiple sclerosis (MS) and related disorders. As an example we have used experimental autoimmune encephalomyelitis (EAE), the most widely used model of MS, since many EAE studies lack evidence of adoption of indicators of quality (Kilkenny et al., 2009; Baker and Amor, 2010; Vesterinen et al., 2010). The guide, reported here, is intended to act as a checklist to aid both authors and referees of manuscripts including the journal Multiple Sclerosis and Related Disorders.

1. Introduction

Animal models of neurodegenerative and inflammatory diseases have greatly contributed to our understanding of human disorders such as multiple sclerosis (MS). These models play a key role in drug development and have led to novel therapeutic approaches to treat human diseases. Nevertheless, some studies showing efficacy of therapies in animal models have not translated well to the clinic. In part, this disparity can be explained by differences in the biology of animals and humans. Another contributing factor is the quality of execution and reporting of studies, which is the responsibility of the authors. However, the acceptance of these papers depends on the quality of refereeing and editorial proficiency. When reporting animal studies, it is recommended that manuscripts conform to the principals of the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines (Kilkenny et al., 2010). This provides a list of 20 guidelines that should be employed in order to make papers consistent as well as transparent. However, conformation to the ARRIVE guidelines requires significantly more information than current publications often report.

We have thus refined the ARRIVE guidelines, incorporated the 3Rs (Reduction, Refinement and Replacement) principals of research with animals, and specifically adapted them to the reporting of animal models of multiple sclerosis (MS) and related disorders. As an example we have used experimental autoimmune encephalomyelitis (EAE), the most widely used model of MS, since many EAE studies lack evidence of adoption of indicators of quality (Kilkenny et al., 2009; Baker and Amor, 2010; Vesterinen et al., 2010). The guide, reported here, is intended to act as a checklist to aid both authors and referees of manuscripts including the journal Multiple Sclerosis and Related Disorders.

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MS, since many EAE studies lack evidence of adoption of indicators of quality (Kilkenny et al., 2009; Baker and Amor, 2010; Vesterinen et al., 2010). The guide, reported here, is intended to act as a checklist to aid both authors and referees of manuscripts, just as the Consolidated Standards of Reporting Trials (CONSORT) guidelines are a compulsory part of reporting clinical trials. Our aim is to improve the conclusions drawn from EAE studies and thus aid better translation to the clinical and treatment of MS.

2. The checklist explained

Importantly, the checklist is not intended to replace the authors’ instructions on the journal websites but recommended as an adjunct to instructions when reporting animal studies. As shown in Table 1 the guide comprises both compulsory elements and points of recommendations. These are current conditions with the aim of gradually phasing-in or modifying the recommended elements to allow adaptations in protocols for EAE and international consensus of the importance of documenting individual items. To aid the refereeing process authors should note the page number of the element in the submitted manuscript.

2.1. Title, abstract, and introduction

As with all manuscripts the title [1] and abstract [2] should accurately reflect the study such that information given does not misrepresent the study. The introduction [3] should contain sufficient background information to allow comprehension of the study and a clear hypothesis [4] with appropriate objectives used to test the hypothesis.

2.2. Materials and methods

To allow reproducibility of studies, often necessary when extending published data, it is crucial to give sufficient information of the materials and the methods. In many EAE studies inclusion of the fine details of the protocol can greatly enhance the quality of the manuscript allowing reviewers, and readers, to understand what was actually carried out. Where controversy arises this may be due to the differences in the EAE protocol. We recommend standardisation of elements of the protocols, including exact details of the antigens and adjuvants used. It is not recommended to use the term complete Freund’s adjuvant (CFA) without specifying the type and dose of mycobacterium used. In addition, immunisation with the myelin peptide of myelin oligodendrocyte glycoprotein (MOG 35–55) is widely used to immunise C57BL/6 mice. However, rarely is consideration given to the conditions under which the peptide is produced. Amino acids have reactive moieties at the N- and C-termini. To minimise side chain reactivity, chemical groups are used to block functional group and, in our hands, impact on the incidence severity of EAE (Amor unpublished data). Therefore it is important to detail for example whether the peptide has an amide of carboxylic acid tail. While international ethical guidelines vary, publication of animal studies requires a statement of the ethical review process [5a]. Within the European Union, animals in research are to be protected by Directive 2010/63/EU as well as national and local legislations. That EAE is regarded as a severe/substantial procedure under this directive; the issue of licences requires strict justification of EAE studies. As yet the checklist does not require justification of EAE studies [5b] but, where ethical review processes are not in place, recommends adherence to the principles of 3Rs. For example, immunisation of C57BL/6 mice induces chronic EAE and is widely-used to examine therapeutic approaches. Once statistical analyses demonstrate an effect the study should be stopped (Figure 1). To aid transparency, and limit bias, details of blinding of the studies, as well as drug/vehicle, and randomisation should be included [6]. In a meta-analysis, where blinding was not performed over-estimation of the efficacy of treatments occurred (Vesterinen et al., 2010). To standardise the procedures, exact routes of administration should be give although details of time of day, and rational for drug doses, are currently optional [7]. In contrast, the details of the animal species and strain in the study must be given and the correct genetic nomenclature (www.informatics.jax.org/mgihome/nomen/) should be used before being abbreviated. The term murine, meaning rat or mouse, should not be used. In the case of transgenic mice, brief details of the breeding and the control animals should be given. It is also recommended that brief details of the housing and conditions of animal maintenance be given [9] since susceptibility to EAE is influenced by e.g. temperature and seasons (Teuscher et al., 2004).

To allow correct interpretation of the data, it is important to perform a power analysis to ensure sufficient numbers of animals in an experimental group [10]. In many ethical applications power analysis is required yet many published EAE studies do not report power analysis (Vesterinen et al., 2010). While this is, as yet, a recommendation for publication, data clearly containing too few animals is not acceptable. Here, it must be remembered that clinical scores used to assess EAE are non-linear (Fleming et al., 2005; Al-Izki et al., 2012) and power calculations for non-parametric statistical analysis must be used. Where the study has been replicated to ensure reproducibility, especially when small numbers of animals are used the replicates should be detailed in both in the text and the figures or legends [10c]. Since EAE studies are frequently used to assess efficacy of therapies it is important that animals are randomly [11] allocated to groups, for example equally distributing males and female animals. Another issue when therapies are initiated after the onset of disease, is to that the outcome response, for example weight loss that can sometimes precede neurological signs of EAE is not segregating before treatment is initiated (Figure 2). Primary outcome measures of most EAE studies are clinical neurological disease [12] for which non-parametric analysis must be performed [13]. These include the Wilcoxon signed rank test, Mann Whitney U test and Kaplan–Meier test, or for comparing more than two groups, the Kruskal–Wallis test should be used (Fleming et al., 2005). Support for the neurological data is pathological studies of the spinal cord. When used the interpretation must accurately reflect the pathology since often demyelination is often a result of axonal damage and not direct myelin damage (Baker et al., 2011). Brain lesions do not typically reflect clinical data in rodents and should not be used to assess or support differences in clinical disease between groups.

2.3. Results

The data should be concise in the text and the figures should be self-explanatory. Animal studies invariably use standardized specific pathogen free inbred rodent strains and baseline data is only recommended [14]. EAE studies in other animals, such as non-human primates may be influenced by infectious agents and under these circumstances the health status must be given. Animal numbers [15] in groups are reported in the text and the figure legends and to assess whether sufficient numbers have been used absolute numbers must be given. Likewise, to assess the reproducibility of the data the measure of deviation is essential [16]. When using non-parametrical analysis the disease score should report and plot the median, and not the mean, score. Figures that do not show error bars will not be considered. As well as a figure, a table or text in the results detailing, day of onset, disease scores and frequency of EAE is necessary, such that the figure can be interpreted. This is especially important if the ‘area under the curve’ is used alone, the
data may be misinterpreted (Figure 3). Averse events [17] are seldom reported in EAE studies and yet are crucial indicators of safety as pre-clinical studies of new therapies prior to phase I clinical trials.

2.4. Discussion

The results should be discussed in relation to the proposed hypothesis and existing literature [18]. While this is essential, it is
also recommended that any potential source of bias and limitations of the models used be reported. Interpretation of the [19] data in relationship to MS, other neurological or related disorders, or human biology should be discussed. However discussion should be realistic to avoid over interpretation. When reporting active EAE studies where an experimental therapy has been administered before disease onset the results should be discussed in terms of therapeutic potential.

2.5. Funding and conflict of interest

Provide details of [20] financial support and conflicts of interest [21] (not listed in the ARRIVE guidelines) as stated in instructions to authors.

3. Conclusion

Similar to the CONSORT guidelines for reporting of clinical trials, reporting and refereeing of EAE studies should be improved. The checklist for submission of manuscripts covers aspects of EAE studies that are essential for publication of high quality manuscripts. Some elements are recommendations...
although it is hoped that these will be considered in the planning and execution of animal studies used to understand MS and related neurological disorders.

Conflicts of interest

None.

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References


