Multiple Sclerosis and Related Disorders 1 (2012) 111–115

Contents lists available at SciVerse ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

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

Sandra Amor [a,b,*], David Baker [b]

[a] Department of Pathology, VU University Medical Centre, 1007 MB Amsterdam, The Netherlands
[b] Neuroimmunology Unit, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, United Kingdom

ARTICLE INFO

Article history:
Received 4 November 2011
Received in revised form
7 January 2012
Accepted 12 January 2012

Keywords:
Multiple sclerosis
Experimental autoimmune encephalomyelitis
EAE
Methods
Reviewing
Reporting

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, 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. It is thus recommended that this checklist be adhered to for both authors and referees of papers submitted to all relevant journals 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

* Corresponding author at: Department of Pathology, VU University Medical Centre, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.
Tel.: +31 20 444 2898.
E-mail address: s.amor@vumc.nl (S. Amor).

2211-0348/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.msard.2012.01.003

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

Table 1
Checklist for reporting experimental animal models of multiple sclerosis and related disorders.

Item		Element	C/R	Page
Title	1	Provide an accurate and concise description of the contents	C	
Abstract	2	Provide an accurate summary, research objectives, including the animal species and strain, key methods, findings, and conclusions	C	
Introduction				
Background	3	a. Include sufficient scientific background (with relevant references) to understand the motivation and context for the study	C	
		b. Explain the experimental approach and rationale	C	
		c. Give details of the animal model, how this addresses the scientific objectives and the relevance to multiple sclerosis	C	
Hypothesis and objectives	4	Clearly describe the specific hypothesis and the objectives used to test the hypothesis	C	
Material and methods				
Ethical statement	5	a. Give ethical review permissions, relevant licences, and national or institutional guidelines for the care and use of animals	C	
		b. Justify the use of animals	R	
Study design	6	a. For each experiment give the number of experimental and control groups	C	
		b. Discuss the steps taken to randomise groups and assessment i.e. blinding	C	
		c. Give the experimental unit (e.g. a single animal, group or cage of animals)	R	
		For complex study provide a time-line diagram or flow chart	R	
Experimental procedures	7	For each experiment and group, provide details of procedures: NB. Studies reporting adjuvants administration into the feet or foot pads will not be accepted	C	
		a. How (e.g. drug formulation, dose, site and route of administration, anaesthesia and analgesia used (including monitoring), surgical procedure, method of euthanasia)		
		b. Details of any specialist equipment and supplier	C	
		c. When (e.g. time of day)	R	
		d. Where (e.g. home cage, laboratory, water maze)	R	
		e. Why (e.g. rationale for route of administration, drug dose)	R	
Experimental animals	8	a. Provide species, strain, sex as well as the age and weight ranges. Do not use 'murine'; use rat or mouse	C	
		b. Provide source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status	C	
		c. For transgenic mice, give the background of the wild type mice to alleviate difference due to breeding	C	
Housing and husbandry	9	a. Type of facility e.g. specific pathogen free; cage or housing; bedding material; number of cage companions	R	
		b. Husbandry conditions e.g. breeding programme, light/dark cycle, temperature, chow, access to food and water, environmental enrichment	R	
		c. Welfare-related assessments and interventions prior to, during, or after the experiment	R	
Sample size	10	a. Number of animals in each study and experimental group	C	.
		b. Explain how the number of animals was arrived at i.e. power analysis	R	
		c. Give the number of replications of each experiment	C	
Allocation to groups	11	a. Give full details of randomisation and matching	C	
		b. Describe the order in which the animals in the different experimental groups were treated and assessed	C	
Outcomes	12	Define outcomes e.g. clinical, pathology, immunology, behavioural changes	C	
Statistics	13	a. Provide details of the statistical methods used for each analysis. Clinical scores for EAE are non-linear and require non-parametric analysis either Wilcoxon rank sum test/Mann Whitney *U* statistics should be used and Kruskal–Wallis test when comparing more than two groups	C	
		b. Specify the unit of analysis for each dataset (e.g. single animal, single neuron)	C	
		c. Describe methods used to assess whether the data met the assumptions of the statistical approach	C	
Results				
Baseline data	14	Give health status of animals e.g. weight, microbiological status, prior to treatment	R	
Numbers analysed	15	a. Report absolute number of animals in each group and analysis e.g. 10/20, not 50%	C	.
		b. Explain why any animals or data were not included in the analysis	R	
		c. Explain the need to keep animals in the study for longer than required to obtain meaningful data	R	
Outcomes and estimation	16	a. Report results for each analysis with the measure of precision (e.g. standard error or confidence interval)	C	
		b. Use statistics as in 13 and do not use area under the curve to compare groups	R	
Adverse events	17	a. Give details of all important adverse events in each experimental group	R	
		b. Describe any modifications to the experimental protocols made to reduce adverse events	R	
Discussion				
Interpretation and scientific implications	18	a. Interpret the results, referring to the hypothesis and objectives and relevant published studies	C	
		b. Comment on the study limitations including any potential sources of bias, limitations of the animal model, and the imprecision of the results	R	
		c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research	R	
Translation	19	Comment on how the findings of this study are relevant and able to be translated to multiple sclerosis or other related disorders	C	
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study	C	
Conflict of interest	21	Disclose conflicts of interests	C	

Adapted from Kilkenny et al. (2010). C=compulsory; R=recommended.

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

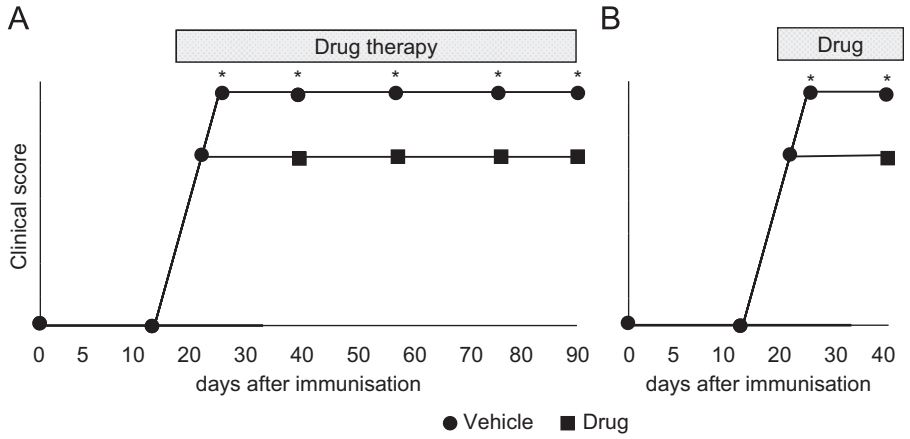

Fig. 1. Reducing the time of the experiment. The impact if the therapy in EAE is already observed by day 25 (A) thus the study can be stopped early in the study (B) to avoid excessive time of animals in an experiment.

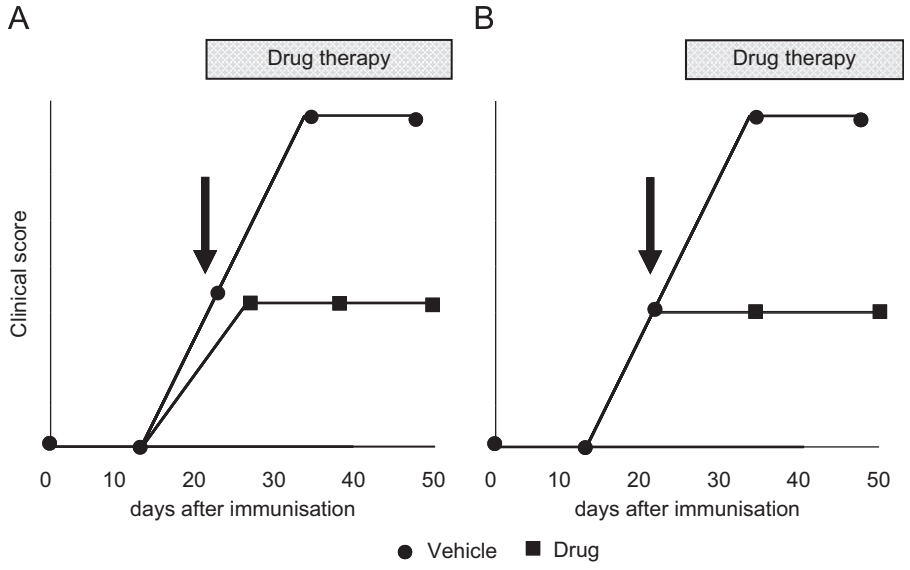

Fig. 2. Randomizing animals in experimental groups. Animals were treated from day 20 post-induction and either non-randomized (A) or randomized (B) into drug or control group. In EAE animals may lose weight before clinical onset potential biasing the study if diseased animals are allocated to the vehicle group (A).

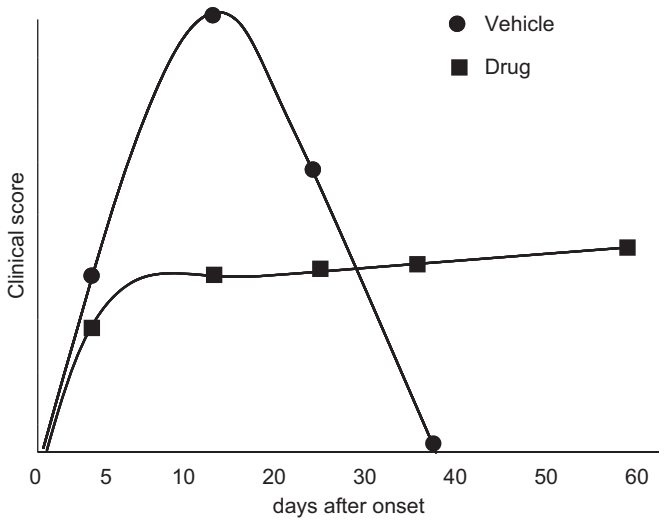

Fig. 3. Analysis using 'area under the curve'. Animals were treated with drug or vehicle and the area under the curve (AUC) used to assess impact on clinical disease. In this study no difference is observed.

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.

Acknowledgements

The authors acknowledge the support from funding agencies in particular the Multiple Sclerosis Society of Great Britain and Northern Ireland, the National Multiple Sclerosis Society, USA, The Stichting MS Research, The Netherlands, AIM2CURE and the National Centre for the Refinement, Reduction and Replacement of Animals in Research.

References

Al-Izki S, Pryce G, O'Neill JK, Butter C, Giovannoni G, Amor S, et al. Practical guide to the induction of relapsing progressive experimental autoimmune encephalomyelitis in the Biozzi ABH mouse. Multiple Sclerosis and Related Disorders 2012;1:29–38.

Baker D, Amor S. Quality control of experimental autoimmune encephalomyelitis. Multiple Sclerosis 2010;16:1025–7.

Baker D, Gerritsen W, Rundle J, Amor S. Critical appraisal of animal models of multiple sclerosis. Multiple Sclerosis 2011;17:647–57.

Fleming KK, Bovaird JA, Moiser MC, Emerson MR, LeVine SM, Marquis JG. Statistical analysis of data from studies on experimental autoimmune encephalomyelitis. Journal of Neuroimmunology 2005;170:71–84.

Kilkenny C, Parsons N, Kadyszewski E, Festing MF, Cuthill IC, Fry D, et al. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. PLoS One 2009;4:e782.

Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biology 2010;8:e1000412.

Teuscher C, Bunn JY, Fillmore PD, Butterfield RJ, Zachary JF, Blankenhorn EP. Gender, age, and season at immunization uniquely influence the genetic control of susceptibility to histopathological lesions and clinical signs of experimental allergic encephalomyelitis: implications for the genetics of multiple sclerosis. American Journal of Pathology 2004;165:1593–602.

Vesterinen HM, Sena ES, ffrench-Constant C, Williams A, Chandran S, Macleod MR. Improving the translational hit of experimental treatments in multiple sclerosis. Multiple Sclerosis 2010;16:1044–55.