STUDY QUALITY IN EXPERIMENTAL STROKE

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Abstract

Background: Poor results in clinical trials for neuroprotectants in acute ischaemic stroke prompt revision of animal data. Meta analysis reveals methodological quality in animal studies is related to estimate of efficacy. In response, animal experimenters state that methodological quality has improved from the past, and that constraints of space lead to under-reporting of operating procedures. I will examine 1) If study quality has changed over time and 2) If study quality is under-stated in publications.

Methods: The data was taken from previous meta-analyses of neuroprotectants in models of acute ischaemic stroke. Data from 426 studies was sorted according to year of publication, split and analysed via non-parametric testing. Corresponding authors of these publications were identified and sent personalised questionnaires in order to detect under-reporting of lab practices.

Results: Mean study quality increased significantly with time. Few important individual components show change with time. 38 responses revealed that some aspects of quality were under-reported.

Conclusions: The components of study quality that have changed with time have little effect in estimate of efficacy. Contacting authors via email and fax is not an effective way to obtain data about methodological quality in animal models of stroke.

Introduction

Animal studies have played a vital part in the evolution of our understanding of the pathobiology of acute ischaemic stroke. The mechanism has been discussed in detail[1] but basically cell damage takes place via several interlinked processes. This yields several therapeutic targets in order to preserve brain function hence reduce symptoms in the aftermath of a stroke. The key concept is the prevention of cell death via action on several possible points on the cascade, such as blockage of calcium channels[2], free radical scavenging[3] and rapid reversal of ischaemia[4] to name a few. Here, all drugs that prevent neuronal death after onset of ischaemia will be termed "neuroprotectants".

The use of animal models of disease has also been criticised[5], mainly owing to disparity of outcome between animal studies and clinical trials in the past. With relation to acute ischaemic stroke, many drugs which have shown efficacy in animal studies have been taken forward to large multi-centre clinical trials, where most have disappointed. This subjected acutely ill patients to undue risk due to wasted time and possible side effects.

This may have happened because of:

1) Non-ideal settings in clinical trials (incorporated both reperfused and non-reperfused patients but mainly reperfusion stroke animal models; different dose; time window)
2) Inadequate power in clinical trials leading to false negatives
3) Different biology in animal models (rat brains show better functional recovery than humans)
4) False positives in animal studies

The challenge of meta-analyses of drugs in animal studies is to obtain an estimate of efficacy based on methodical search strategies of the literature, whilst identifying sources of bias leading to false positive results and ultimately offering better translation between animal studies and clinical use.

Fig. 1: CAMARADES 10 item quality checklist

- **Publication in a peer reviewed journal**
- **Control of temperature**
- **Random allocation to group**
- **Blinded induction of ischaemia**
- **Blinded assessment of outcome**
- **Use of anaesthetic without marked neuroprotective activity**
- **Use of co-morbid animals**
- **Sample size calculation**
- **Compliance with animal welfare regulations**
- **Statement of potential conflicts of interest**

For example, meta-analyses of animal experiments of candidate neuroprotectant FK506 reveals a possible explanation for the overestimation of efficacy is poor methodology of animal experiments[6]. Study methodology has been scored from 1 to 10 against previously published criteria[7,8] (Fig. 1), and stratification of the studies according to study quality score shows a relationship where estimate of efficacy falls with increasing study quality[7].

In light of this data, animal experimenters have stated that: 1) Animal studies in the past may have been of poor quality, but have improved, and 2) Constraints of space lead to underreporting of certain aspects of quality in published articles that the reader would have to assume were performed.

So we will analyse:

1) How mean study quality has changed over time
2) How individual study quality components have changed over time
3) If reported study quality differs from study quality obtained by contacting journal authors

Methods

Data on study quality was obtained from a Microsoft Access database compiled from previous animal meta-analyses of neuroprotectants in temporary and permanent focal models of acute ischaemic stroke. Since publication of the 10 item checklist, further aspects of quality that may have a bearing on effect size have been debated. Two items in question include control of blood pressure and blood gases of the animal during induction of ischaemia, and confirmation of ischaemia via Laser Doppler Flowmetry (LDF). For completeness the 482 publications, of which either hard copies or electronic copies were available, were re-examined and the abovementioned points added to the database.

For analysis the data from 482 publications was split into quartiles by year of publication and analysed as four groups. The mean study quality score in each group was obtained and analysed with a non-parametric Kruskal-Wallis test. When analysing individual components of study quality the proportion of people who reported performance of each respective item was analysed across the 4 groups. Significance was determined by non-parametric Chi-squared testing. Professional statistical advice was sought when required.

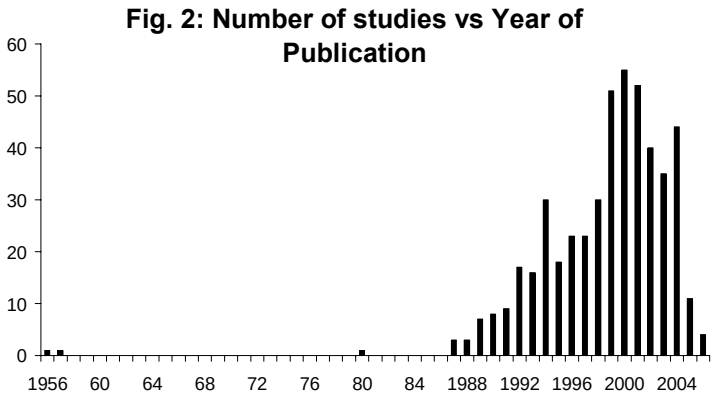
To determine whether contacted authors had performed more items on our study quality checklist than was stated in their published manuscript, corresponding authors that were available via email or fax were contacted. We attempted to obtain a complete set of email addresses or fax numbers by searching the internet for the corresponding authors who had not provided this data. In total 234 corresponding authors with these details were identified and 263 personalised questionnaires (one per publication) were designed. The questionnaire was tailored according to the data already provided in the published article. This was performed using a mail merge function in MS-Word. (Appendix 2)

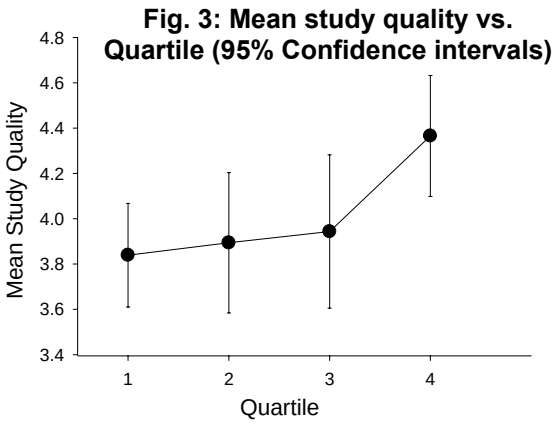
Email was the preferred method, and faxes were sent to those who had not provided an email address. When emails bounced back, the most recent e-mail address for that corresponding author was searched for on the internet and the questionnaire resent.

Owing to poor response rate following 3 weeks, faxes were sent to all corresponding authors for whom we could find a fax number. An email-to-fax-merge was performed using FaxforWord ver. 1.1.5. (Addins for Office). 188 faxes were sent, however no additional authors were contacted.

Pairing the response for each quality aspect (i.e. either “yes” or “no”) with its counterpart in the database allowed for a simple paired analysis. This generated the frequency with which authors would state that they performed an aspect of quality that was not reported in the published manuscript.

Results



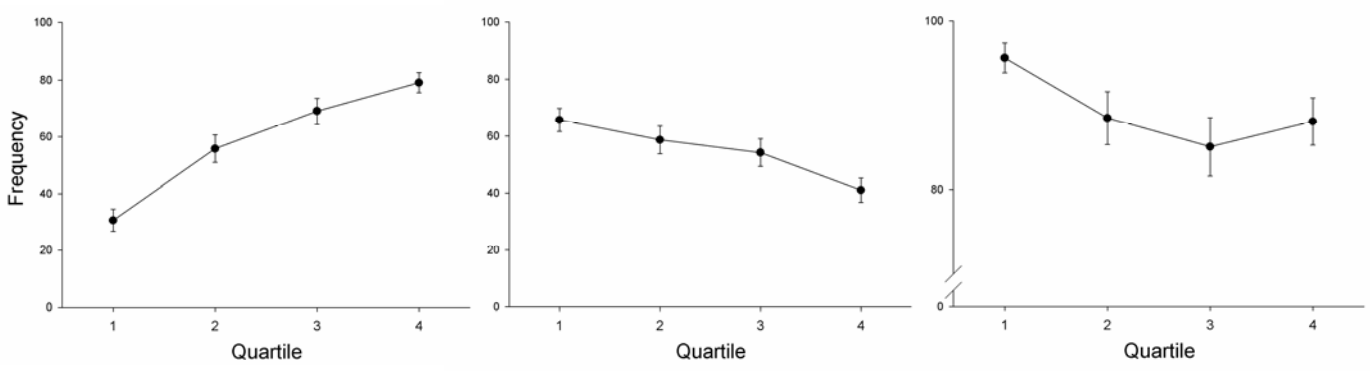


Change of study quality over time

All 482 publications were included in the analysis. The number of studies each year varied, and the majority were clustered in the last 20 years. (Fig 2)

The quartiles were numbered 1 to 4 from earliest to latest studies by year. The mean quality score across quartiles 1, 2, 3 and 4 (with 95% confidence intervals) was 3.84, 3.89, 3.94 and 4.37. The rise with time was significant (χ^2 = 12.831; p = 0.005) (Fig 3). The median score (inter-quartile range) was 4 (2-6), and did not change across the groups.

Analysis of certain individual components revealed change over time. The number of comparisons where compliance with animal welfare regulations was carried out increased with time from 30.7% to 79.1%. Control of physiological variables and publication in a peer reviewed journal decreased with time from 65.7% to 41% and 95.6% to 88.1%. (Fig 4) The other variables did not change significantly with time.



Figs. 4a, 4b, 4c respectively. Number of comparisons where Compliance with animal welfare (χ^2 = 72.272; p<0.0005), control of blood pressure and blood gases (χ^2 = 17.436; p<0.001), and publication in a peer-reviewed journal (χ^2= 8.213; p = 0.042) were carried out. Error bars are standard error of proportion.

Over all quartiles the percentage of studies that performed each aspect on the quality score checklist varied considerably. (Table 1)

Aspect of Quality	*% of comparisons*
Peer-reviewed	89.6%
Control of Temperature	81.1%
Random Allocation to group	30.3%
Blinded ischaemia	8.1%
Blinded outcome assessment	26.1%
Anaesthetic non neuroprotective	78.4%
Use of co-morbid animals	13.1%
Animal welfare regulations	58.1%
Sample size calculation	1.7%
Conflicts of interest	15.6%
Control of physiological variables	54.8%
Laser-Doppler Flowmetry	19.7%

Table 1: Performance on each aspect of quality in all 4 quartiles

Responses

70 of the 263 questionnaires could not be sent. Results from 2 authors are still pending at the time or writing. We received responses from 23 authors, and some who had received more than one questionnaire responded on the basis that answers would apply to all publications. This resulted in 38 responses (i.e. a 19.7% response rate) with "corrected" quality items. The frequency with which authors stated they performed certain aspects of quality not reported in the published manuscript (± 95% Confidence Intervals) was: control of temperature 18.42% (±13.65%); control of physiological variables 42.11% (± 10.53%); randomisation to group 2.63% (±13.65%); blinded induction of ischaemia 47.37% (±21.88%); blinded assessment of infarct volume 50% (±1.38%); blinded assessment of neurological score 2.63% (± 31.79%); sample size calculation 36.84% (±19.3%); and no responders stated they confirmed ischaemia via LDF that was not already reported in the publication.

Discussion

A number of conclusions can be made from the results.

1) Overall reported study quality has increased but not by much.

2) What has increased has very little influence in the estimate of efficacy.

3) Reporting of some quality aspects have decreased significantly with time.

The improvement seems largely due to the increase in compliance with animal welfare regulations. Performing animal experiments in a humane fashion is not strictly a quality aspect on its own; however we feel it reflects overall lab practices. Pilot data however shows that it does not affect estimate of efficacy.
Control of physiological variables significantly decreased with time- therefore this practice is either reducing in prevalence or has become standard and are being under-reported owing to space constraints.
Why peer-reviewed publication may decrease with time may be explained by time delay between publishing in a peer-reviewed article and indexing in the search engines used for the meta-analyses.
It is difficult to disentangle the variation of total study quality in time from the fact that we have more abstracts (hence more "incomplete" publications) now than before, and indeed some reviewers would prefer to analyse peer- reviewed publications only[9]. However, a post-hoc analysis of study quality in peer-reviewed journal articles with the abstracts filtered out from the analysis revealed no other significant differences across the 4 quartiles.
Pilot data has revealed that the aspect of study quality corresponding with the largest increase in estimate of efficacy is the use of ketamine anaesthesia, although it has been argued that other anaesthetics also possess some neuroprotective properties[10,11,12]. In any case, this particular aspect did not change significantly ($\chi^2 = 5.123$; $p = 0.163$) over each group.

There are several quality scores that have been used for the assessment of animal study methodology. The 10 item checklist used is based on recommendations covered by the Stroke Therapy Academy Industry Roundtable (STAIR)[13]. However certain aspects of the STAIR criteria cover the range of data that can be obtained via experimentation (e.g. dose-response curves, administration of drugs on different animal models and at different time points). It may be argued here the use of co-morbid animals, is an aspect of the range of data rather than quality.
Van der Worp et al[14] and Horn et al[15] have used a combination of aspects from the CAMARADES checklist as well as the STAIR criteria. Stratifying animal studies according to the CAMARADES quality score however has been proven to show a relationship between estimate of efficacy of a drug and its quality score as mentioned.
The disadvantage to using any quality score is that different components will have unequal bearing on effect size; therefore 2 studies with the same score of "4/10" may be completely different in terms of actual quality of the study (See *Further work*).

Questionnaires

It is clear that contacting authors via this method does not yield the best results for assessing quality in studies since response rates are so low. Interestingly, post-hoc analysis revealed that the mean quality score of the responders was significantly higher than average. (Means difference 0.66; 95% CI 0.130 to 1.197; t=-2.445; p=0.015 assuming equal variances). Caution is required in interpretation of the results.

The responses to date would indicate that control of temperature, control of physiological variables, blinded induction of ischaemia, blinded assessment of infarct volume and performing a sample size calculation prior to the experiment are under-reported in publications. However the number of replies is few and some responses based on identical lab practices being performed for all publications from one laboratory, which may not be the case in truth. We may be seeing the beginnings of important results but cannot yet make any conclusions based on the replies to date.

In order to increase response rate and get as much meaningful data from each author, the items of our questionnaire were personalised depending on data already available in the publication. For example, if the published manuscript mentioned randomisation to treatment, item 3 on the questionnaire would state "You mentioned that allocation to treatment group was randomised. Could you confirm the method used?", followed by a list of choices. (Appendix 1.1) The same item on a questionnaire sent to an author whose paper did not mention randomisation specifically would initially ascertain if treatment allocation was randomised (Appendix 1.2).

Every item in the questionnaire was chosen either because the quality item was not mentioned in the published manuscript, or because further knowledge into the methods used by a laboratory would give us insight into the standard operating procedures employed. The potential for bias to be introduced at each stage of the experimental procedure has been discussed[16], with a possible effect on result. Where possible, through the questionnaire we tried to elicit exclusion criteria for control of variables such as temperature, blood gases and blood pressure beyond which the animal was discarded. These are by and large not mentioned in such studies[17]. There is need for controlling temperature amongst other physiological variables, since we would wish to know if the drug was effective owing to its neuroprotectant properties, or because it affected such variables in a way beneficial to the animal. It was noted that some respondents indicated that such variables were monitored in preliminary experiments and were found not to deviate from set criteria, and therefore there was no necessity to carry out similar monitoring in subsequent experiments.

There is opportunity for the experiment to be biased should the surgeon inducing ischaemia know which treatment group the animal is in, or if the surgeon gained experience performing surgery on the control animals before operating the animals meant for the treatment[18]. In the questionnaire we elicited the method of randomisation, with some surprising results. The list of choices for method of randomisation in our questionnaire contained some options that by clinical trial standards wouldn't be adequate at all. We were surprised to find such a large number of respondents selecting "animals randomly chosen from a cage" as their method of randomisation. This may not be a fair criticism, considering the limited numbers of lab staff. However it is a possible source of bias since "weaker" animals may be easier to catch from a cage. In the defence of the majority, they did not declare randomisation in their paper; however one

respondent having done so, selected this "non-randomisation" option from the list of choices. One such respondent also chose "coin toss", which is not true randomisation either. In a response, a professor of neurosurgery in a prestigious American institution commented that there was no need for such practices if the research group was "experienced or honest", and they are done more in studies among pharmaceutical companies. He also stated "there is no way of predicting the outcome despite a study hypothesis". It is also interesting to note that some studies quote the phrase "double-blinding" in the published manuscript of an animal experiment (i.e. both researcher and animal did not know the treatment group). The sentiment shown here and the incorrect use of this jargon are worrying at best.

It has been argued that a sample size should be determined *a priori* that would give the experiments sufficient statistical power to detect a reasonable effect size. It should be noted that while majority of the respondents stated that "Investigator Experience" was enough to determine a sample size, in the majority of studies there is simply not enough statistical power to detect a reasonable effect size[19]. The quality score gives a point for a predetermined sample size regardless of what it is, which has been criticised[20], however it gives the experimenting group merit for sticking to clinical trial protocol as closely as possible, leaving aside issues with funding (cost of animals). It may seem from the results of the questionnaire that more people perform these calculations than is reported in the journals.

One of the main complaints of publishing conflict of interest in animal studies is that they are often submitted for publication but left out by the editors of the journal in the manuscript. Therefore the questionnaire did give the authors a chance to declare such matters, and although majority of respondents simply stated that none existed, one major group stated they owned the patent for nicotinamide in stroke and declared this to the journal, but the statement was not published.

Overall, analysis of each quality aspect revealed a vast variation in the reporting of certain practices. (Table 1) This may reflect importance placed on individual quality items by different review groups, or just differences in practicality of performing these. Should pre-clinical trials be thought of as a stepping-stone to clinical research, every effort must be made to reduce bias in experiments. In order to do so, emphasis has to be placed on these aspects of study quality.

Limits of my methodology

The data obtained was from a database of very thoroughly searched systematic reviews.[21,22,23,24,25,26,27,28] Obtaining data from a source other than the primary source may have increased the possibility of transcription errors. Even though the data was obtained from as early as 1956[29], studies over 20 years old are few. This could be to a number of reasons- mainly that 1) Recent interest in neuroprotectant studies in stroke may have increased substantially, and 2) The meta-analyses that made up my data source were carried out recently. Each meta-analysis looked at an individual drug or drug group, most probably one

that was under thorough experimentation recently. Since this was pertaining to change in quality over time, having study quality data for a complete set of animal experiments in acute ischaemic stroke may have had a significant bearing on the results. In addition to this elements of publication bias that may have led to the overestimation of drug efficacy.

The questionnaire design may be improved upon. The emailed version of the questionnaire could have had functional tick-boxes, thereby making them quicker and easier to fill out. Designing this was beyond my capability however. A published study of how the questionnaire could flatter readers to increase response rate[30] came up with some useful suggestions but any alterations at the time would have been late.

The number of responses was limited by the fact that some authors had changed location whose contact details could not be found via further searching.

Further Work

To make the study quality score best represent quality of work in a lab it would need refining to give a best estimate as to how methodology would effect estimate of efficacy. Thus a quality score with different weighting on components is much in need.

Contacting authors via post is likely to yield a better response rate[30] than emails and far more publications include a postal correspondence address. This would be next if we were to exhaust this method of procuring quality data completely.

Another suggested method is a central database of standard operating procedures set up online, which could be incorporated as a necessity for licensing. A central database with up-to-date contact details of authors would be a start.

Acknowledgements

I would like to thank Dr. Malcolm Macleod and Ms. Emily Sena for their continuous support and feedback. I would also like to thank Mr. Chris Matthews at the IT section of the Department of Clinical Neurosciences for help with the email-to-fax merge, Drs. Maggie Lai and David Howells for their feedback with the questionnaire, and Dr. Margaret MacDougal for her assistance with the statistical methods used in this project. I am also grateful to the staff at DCN for the opportunity to present my work.

Word Count : 2990 (Excluding abstract, tables, figures and legends)

Matric: 0237869 13/11/2006

Appendix 1.1

Rainer Kollmar
Univeristy of Hieldelberg

Dear Prof/Dr Kollmar ,

We are a research team based at the University of Edinburgh. We are investigating how aspects of study design, conduct and reporting might affect the estimate of how effective drugs are at reducing infarct volume in animal models of focal cerebral ischemia. One important consideration is that important factors may be omitted from published reports because of constraints of space or the decisions of editors.

We have previously identified your publication *Kollmar R, Henninger N, Bardutzky J, Schellinger PD, Schäbitz W-R, Schwab S. Combination therapy of moderate hypothermia and thrombolysis in experimental thromboembolic stroke--an MRI study. Exp Neurol 2004;190:204-212.* in our meta-analysis of the efficacy of Hypothermia. We would be very grateful for your assistance in completing the following, brief questionnaire, which aims to establish how closely our reading of your published work reflects what you actually did. For each section, please indicate all responses which apply, and feel free to add comments as you consider appropriate.

We are conscious that we may have made errors in our interpretation of your written work. If this is the case, please accept our apologies, and indicate the nature of our errors on your questionnaire or accompanying email.

We have sent a questionnaire for every publication we identified which came from your lab; if the same methods were used across all publications then please let us know, and return only one completed form. However if the methods used were different it would be extremely helpful to us for you to complete a questionnaire for each set of methods, indicating to which publications they refer.

We realise that there are a lot of competing demands on your time, and to reflect this one respondent (drawn at random from those of the 252 scientists sent a questionnaire who return it) will receive a $100 Amazon gift voucher to contribute towards the purchase of for instance textbooks for your lab.
You may return the questionnaire by email, by fax or by post to the address below.

We thank you in advance,

Shehan Samaranayake Malcolm Macleod
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Fax: +44131 3325150
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University of Edinburgh
Western General Hospital
Crewe Road,
Edinburgh
EH4 2XU UK

Questions

Please tick where appropriate

1. Temperature Control

You mentioned that you measured temperature during the experiment.

Was body temperature measured ? ☐

Was brain temperature measured ? ☐

If temperature deviated from pre-set values did you take action to return it to normal?

Yes ☐ No ☐

If YES What criteria did you use?.....

What did you do?.....

If temperature deviated from pre-set values did you exclude that animal from further analysis?

Yes ☐ No ☐

If YES What criteria did you use?

2. Control of physiological variables

Was **mean arterial blood pressure** measured during the surgery?

Yes ☐ No ☐

How was it measured?

Tail Cuff ☐

Pressure sensitive catheter ☐

Telemetry ☐

Other method (Please Specify) ……

If BP deviated from pre-set criteria did you take action to return it to normal?

Yes ☐ No ☐

If YES What criteria did you use? ……

What did you do?.....

If BP deviated from pre-set criteria did you exclude that animal from further analysis?

Yes ☐ No ☐

If YES What criteria did you use? ……

Were **blood gases** measured during the surgery?

Yes ☐ No ☐

How were they measured ?

Capilliary gases ☐

Expired gases ☐

Oximetry ☐

Other method (Please specify) ……..

If blood gases deviated from pre-set criteria did you take action to return them to normal?

Yes ☐ No ☐

If YES What criteria did you use?.....

What did you do?.....

If blood gases deviated from pre-set criteria did you exclude that animal from further analysis?

Yes ☐ No ☐

If YES What criteria did you use?.....

3. Allocation to group

The manuscript indicates that the animals were randomly allocated to a group. What method of randomisation did you use ?

Computer generated treatment schedule ☐

Random number tables ☐

Animals randomly chosen from the cage ☐

Coin toss ☐

According to date or days of the week ☐

Other (Please describe) ……

4. Induction of Ischaemia

The manuscript does not indicate whether ischemia was performed without the surgeon knowing the treatment group to which the animal belonged. Was this the case?

Yes ☐ No ☐

If so, how was this acheived ?

- Surgeon remained blinded to group allocation throughout ☐
- Drug administered by another individual ☐
- Drug administered using coded solutions ☐
- Group allocation held securely (eg sealed envelopes) until after ischaemia induced ☐
- Group allocation held securely (eg sealed envelopes) until after ischaemia completed ☐
- Group allocation determined after onset of ischaemia ☐
- Other (Please specify) …….

5. Confirmation of Ischemia

Did you have any criteria for selecting only animals in which ischaemia was successfully induced?

Yes ☐ No ☐

If YES, what did you use ?

- Laser Doppler flow? ☐
  - If so, what criteria? …….
- Development of neurological signs? ☐
  - If so, which ones? ……...

Other Technique (Please specify) ……..

6. Assessment of Outcome

In your publication you report that the scientist(s) responsible for assessing **infarct volume** was blinded to which group the animals belonged to. How was this achieved?

Fully automated computer system ☐
Partially automated computer system ☐
Slides re-labeled with code numbers ☐
Assesor unaware of treatment allocation ☐
Slides presented in random order ☐
Different assesor with no prior knowledge of treatment group ☐
Other (Please specify) .....

7. Sample size

The published article does not describe whether the number of animals to be used was detrmined in advance, or whether group sizes were increased to increase statistical power. Was the final sample size determined in advance?

Yes ☐ No ☐

Is so, was this based on

- Investigator experience ☐
- Formal sample size calculation ☐

8. Conflict of Interest

The publication does not describe any potential conflict of interest. Was this because

A potential conflict of interest was declared to the publisher during the review process, but they did not include this with the published version ☐

.

You have a potential conflict of interest but did not declare this since it was not considered of sufficient magnitude to merit declaration. ☐

No potential conflict of interest existed at the time of publication. ☐

Thank you very much for your time. We'd be happy to respond to any queries at the email address above.

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Appendix 1.2

E Hanon
University of Liege

Dear Prof/Dr Hanon ,

We are a research team based at the University of Edinburgh. We are investigating how aspects of study design, conduct and reporting might affect the estimate of how effective drugs are at reducing infarct volume in animal models of focal cerebral ischemia. One important consideration is that important factors may be omitted from published reports because of constraints of space or the decisions of editors.

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We thank you in advance,

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If YES What criteria did you use?.....

What did you do?.....

If temperature deviated from pre-set values did you exclude that animal from further analysis?

Yes ☐ No ☐

If YES What criteria did you use?

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Was **mean arterial blood pressure** measured during the surgery?

Yes ☐ No ☐

How was it measured?

Tail Cuff ☐

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Telemetry ☐

Other method (Please Specify) ……

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Yes ☐ No ☐

If YES What criteria did you use? ……

What did you do?.....

If BP deviated from pre-set criteria did you exclude that animal from further analysis?

Yes ☐ No ☐

If YES What criteria did you use? ……

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Yes ☐ No ☐

How were they measured ?

Capilliary gases ☐

Expired gases ☐

Oximetry ☐

Other method (Please specify) ……..

If blood gases deviated from pre-set criteria did you take action to return them to normal?

Yes ☐ No ☐

If YES What criteria did you use?.....

What did you do?.....

If blood gases deviated from pre-set criteria did you exclude that animal from further analysis?

Yes ☐ No ☐

If YES What criteria did you use?.....

3. Allocation to group

The manuscript does not indicate the method of allocation to group. How were the animals allocated?

Random allocation ☐

Alternate animals ☐

Batch by Batch ☐

Other (please specify) .....

If it was random allocation, what was the method of randomisation used ?

Computer generated treatment schedule ☐

Random number tables ☐

Animals randomly chosen from the cage ☐

Coin toss ☐

According to date or days of the week ☐
Other (Please describe) …….

4. Induction of Ischaemia

The manuscript does not indicate whether ischemia was performed with the surgeon blinded to the treatment group. Was this the case?

Yes ☐ No ☐

If so, how was this acheived ?

Surgeon remained blinded to group allocation throughout ☐
Drug administered by another individual ☐
Drug administered using coded solutions ☐
Group allocation held securely (eg sealed envelopes) until after ischaemia induced ☐
Group allocation held securely (eg sealed envelopes) until after ischaemia completed ☐
Group allocation determined after onset of ischaemia ☐
Other (Please specify) …….

5. Confirmation of Ischemia

Did you have any criteria for selecting only animals in which ischaemia was successfully induced?

Yes ☐ No ☐

If YES, what did you use ?

Laser Doppler flow? ☐
If so, what criteria? …….
Development of neurological signs? ☐
If so, which ones? ……...

Other Technique (Please specify) ……..

6. Assessment of Outcome

It is not clear from your publication whether the scientist(s) responsible for assessing **infarct volume** were blinded to which group the animals belonged. Was this the case?

Yes ☐ No ☐

If YES could you confirm how this was achieved?

Fully automated computer system ☐
Partially automated computer system ☐
Slides re-labeled with code numbers ☐
Assesor unaware of treatment allocation ☐
Slides presented in random order ☐
Different assesor with no prior knowledge of treatment group ☐
Other (Please specify) .....

7. Sample size

The published article does not describe whether the number of animals to be used was detrmined in advance, or whether group sizes were increased to increase statistical power. Was the final sample size determined in advance?

Yes ☐ No ☐

Is so, was this based on

Investigator experience ☐
Formal sample size calculation ☐

8. Conflict of Interest

The publication does not describe any potential conflict of interest. Was this because
A potential conflict of interest was declared to the publisher during the review process, but they did not include this with the published version ☐

Matric: 0237869 13/11/2006

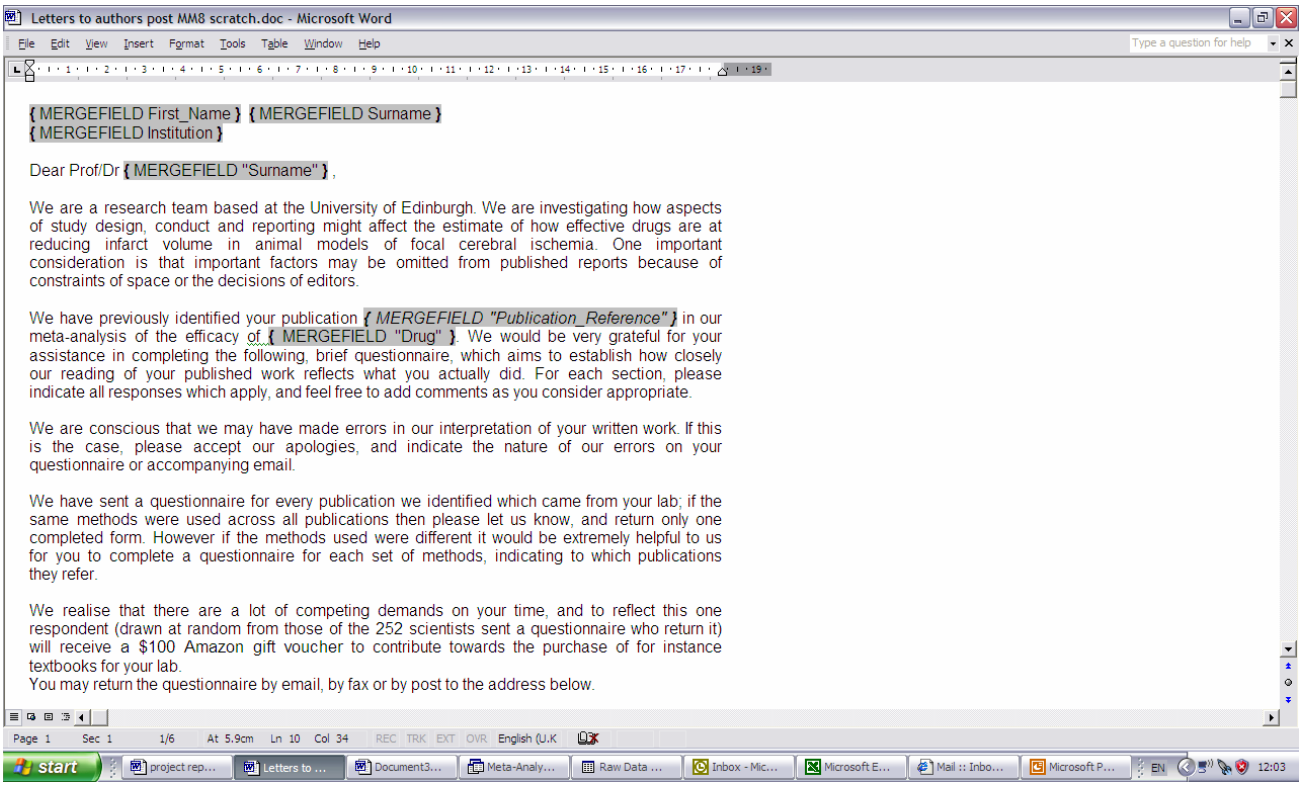
.

You have a potential conflict of interest but did not declare this since it was not considered of sufficient magnitude to merit declaration. ☐

No potential conflict of interest existed at the time of publication. ☐

Thank you very much for your time. We'd be happy to respond to any queries at the email address above.

Appendix 2



Letters to authors post MM8 scratch.doc - Microsoft Word

{ MERGEFIELD First_Name } { MERGEFIELD Surname }
{ MERGEFIELD Institution }

Dear Prof/Dr { MERGEFIELD "Surname" } ,

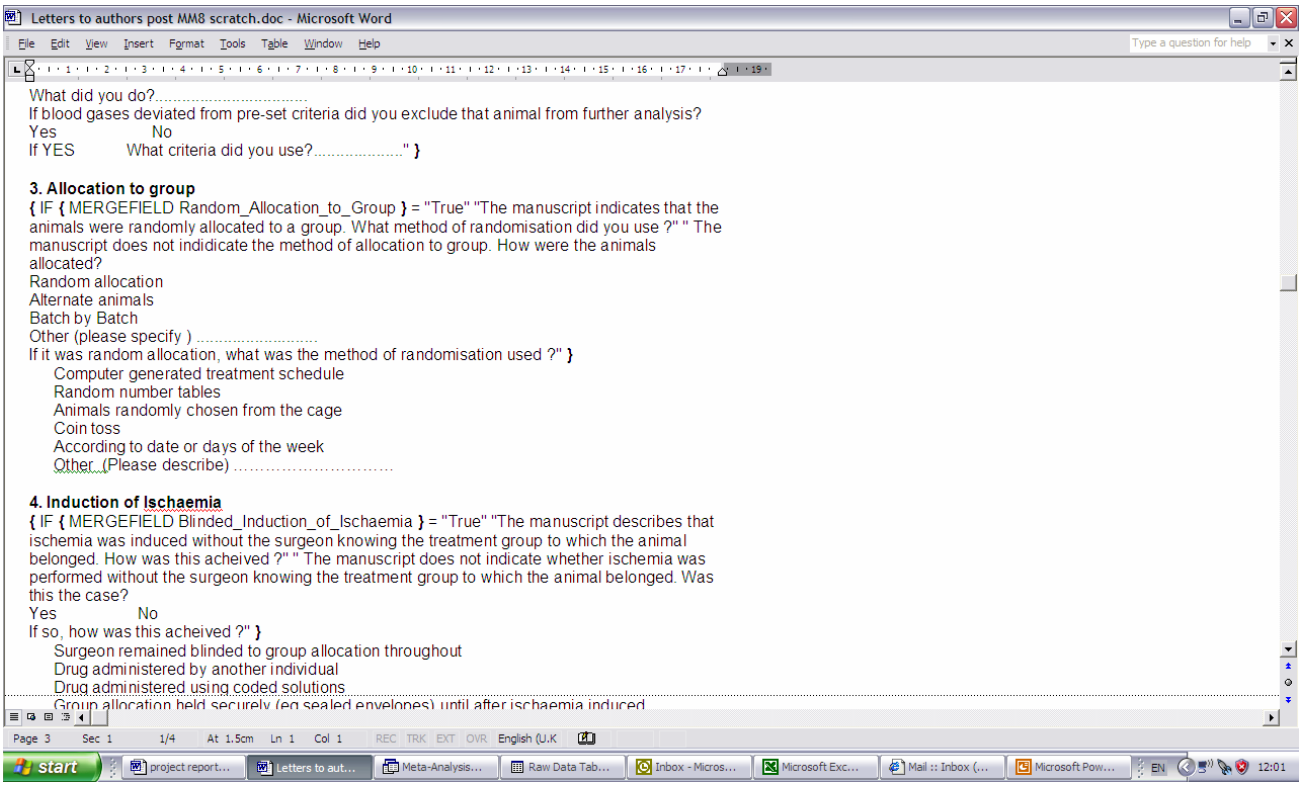
We are a research team based at the University of Edinburgh. We are investigating how aspects of study design, conduct and reporting might affect the estimate of how effective drugs are at reducing infarct volume in animal models of focal cerebral ischemia. One important consideration is that important factors may be omitted from published reports because of constraints of space or the decisions of editors.

We have previously identified your publication *{ MERGEFIELD "Publication_Reference" }* in our meta-analysis of the efficacy of { MERGEFIELD "Drug" }. We would be very grateful for your assistance in completing the following, brief questionnaire, which aims to establish how closely our reading of your published work reflects what you actually did. For each section, please indicate all responses which apply, and feel free to add comments as you consider appropriate.

We are conscious that we may have made errors in our interpretation of your written work. If this is the case, please accept our apologies, and indicate the nature of our errors on your questionnaire or accompanying email.

We have sent a questionnaire for every publication we identified which came from your lab; if the same methods were used across all publications then please let us know, and return only one completed form. However if the methods used were different it would be extremely helpful to us for you to complete a questionnaire for each set of methods, indicating to which publications they refer.

We realise that there are a lot of competing demands on your time, and to reflect this one respondent (drawn at random from those of the 252 scientists sent a questionnaire who return it) will receive a $100 Amazon gift voucher to contribute towards the purchase of for instance textbooks for your lab.
You may return the questionnaire by email, by fax or by post to the address below.



Letters to authors post MM8 scratch.doc - Microsoft Word

What did you do?.....
If blood gases deviated from pre-set criteria did you exclude that animal from further analysis?
Yes No
If YES What criteria did you use?....." }

3. Allocation to group

{ IF { MERGEFIELD Random_Allocation_to_Group } = "True" "The manuscript indicates that the animals were randomly allocated to a group. What method of randomisation did you use ?" " The manuscript does not indidicate the method of allocation to group. How were the animals allocated?
Random allocation
Alternate animals
Batch by Batch
Other (please specify) .....
If it was random allocation, what was the method of randomisation used ?" }
Computer generated treatment schedule
Random number tables
Animals randomly chosen from the cage
Coin toss
According to date or days of the week
Other (Please describe) .....

4. Induction of Ischaemia

{ IF { MERGEFIELD Blinded_Induction_of_Ischaemia } = "True" "The manuscript describes that ischemia was induced without the surgeon knowing the treatment group to which the animal belonged. How was this acheived ?" " The manuscript does not indicate whether ischemia was performed without the surgeon knowing the treatment group to which the animal belonged. Was this the case?
Yes No
If so, how was this acheived ?" }
Surgeon remained blinded to group allocation throughout
Drug administered by another individual
Drug administered using coded solutions
Group allocation held securely (eg sealed envelopes) until after ischaemia induced

Matric: 0237869 13/11/2006

PERSONAL REVIEW

Project Objectives

To determine if study quality in animal models of acute ischaemic stroke :

1. Has improved over time as authors of such studies claim
2. Is under-stated in publications due to constraints of space, thus calling into question the current method of reading of journal articles in order to obtain study quality
3. Determine if direct contact with authors is a better method of obtaining data for study quality

Personal objectives

My current knowledge of statistical methods, presentation skills, and knowledge of communication methods with journal authors are limited. My aims of this project were to improve on these. My skills in critical appraisal of journal articles could be improved upon, and the numbers of articles I had to read in a short space of time meant had ample opportunity to improve my technique in this area.

Abstract

Background: Poor results in clinical trials for neuroprotectants in acute ischaemic stroke prompt revision of animal data. Meta analysis reveals methodological quality in animal studies is related to estimate of efficacy. In response, animal experimenters state that methodological quality has improved from the past, and that constraints of space lead to under-reporting of operating procedures. I will examine 1) If study quality has changed over time and 2) If study quality is under-stated in publications.

Methods: The data was taken from previous meta-analyses of neuroprotectants in models of acute ischaemic stroke. Data from 426 studies was sorted according to year of publication, split and analysed via non-parametric testing. Corresponding authors of these publications were identified and sent personalised questionnaires in order to detect under-reporting of lab practices.

Results: Mean study quality increased significantly with time. Few important individual components show change with time. 38 responses revealed that some aspects of quality were under-reported.

Conclusions: The components of study quality that have changed with time have little effect in estimate of efficacy. Contacting authors via email and fax is not an effective way to obtain data about methodological quality in animal models of stroke.

Project review

Obtaining data on study quality from publications is suspect. It is the opinion of many senior authors of journal articles that lab practices are sometimes *over*stated and not understated as animal experimenters claim. This would also throw into doubt the answers provided in questionnaires – which may be even less reliable! I do not wish to question the honesty and integrity of members in the research community, however one must bear in mind that we are assuming that everyone is being honest.

Before sending off the questionnaires I overestimated the response rate I would get. I did not foresee difficulties with emails not being sent, and authors having moved. This presented additional difficulty, and occurred when the end of the project was drawing near. Finding contact details for prominent authors in the field over the internet is harder than it seems. Indeed many authors of publications were left out for this reason.

Additionally, barriers in language may have made some of the questions difficult to interpret to some foreign authors - this was not reflected in any of the answers.

Personal review and statement

My skills with handling statistics and preparing a presentation have improved. I feel these skills will benefit me in future. I could not overly familiarise myself with all the statistical packages I used (i.e. SPSS and SigmaPlot), however I feel I have been given a good introduction. Unexpectedly, my skills with *four* Microsoft applications (Word, Excel, Powerpoint and Access) have also improved. I learnt to manage my time effectively, and learnt to communicate concisely over email. I was not taxed until the end of the project, when the questionnaire was finally designed and unforeseen problems occurred following the mail and fax merges.

All in all, the experience was rewarding.

Estimation of Grade

a) Performance – B
b) Written report – B
c) Overall mark – B

The nature of the work I had to do for this project was different to what I had experienced before. I felt I had to learn new skills in a short space of time, and patiently use software I was not familiar with. I have tried to best fit my data with what is already known on the subject of study quality in these kind of experiments and I hope my report is reflective of the time put on it.

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