Letters to the Editor

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Criteria for Valid Preclinical Trials Using Animal Stroke Models

The editorial by Hsu1 presents rigorous criteria for valid animal studies of stroke treatments. These criteria could also be advocated for all animal experiments. Consequently, a careful examination of the cost-benefit relationship of his recommendations is in order. Dr Hsu is concerned about observer bias and about bias by the person producing an intervention (eg, carotid ligation). He believes unconscious bias could influence outcome if the person measuring end points knows the treatment or if the performer of the intervention knows whether the animal will be treated. In human studies there are two reasons for “blind” doctors. Not only may their bias affect their measurements, but through a placebo effect, it may actually influence the performance of the patient. The placebo effect is not a factor in animal studies. Consequently, we have only the risk of observer bias to worry about. In most instances this can be avoided by objective end points supplemented at least initially by ignorance on the part of the observer concerning “expected” or hypothesized outcome.

In histological studies complete observer blindness can be maintained without hiring additional staff, simply by coding slides. For physiological studies there would often be the expense of hiring staff to make observations with no knowledge of treatment or hypothesis concerning drug effect. The unconscious systematic bias-driven procedural errors (eg, giving “short-weighted” injections; ligating vessels partially rather than completely) seem rather far-fetched to me. In the case of stroke models, an objective measurement of flow immediately after ligation would certainly reveal biased preparation of animals and is a mandatory part of the experimental design. Dr Hsu has a rather cavalier approach to current funding problems. He says that since large sums are already spent for human trials, the National Institutes of Health ought not balk at expanded spending for the animal experiments to enable us to hire the additional staff required for blinded studies. This is simply unrealistic. Investigators should eliminate as many opportunities for bias as possible. However, because we do not have to worry about a placebo effect, the simple measures outlined above should suffice to ensure valid data.

Valid data are robust data; that is, they can be reproduced by others. Often editors and reviewers downgrade manuscripts for not being original. In fact, reproducibility is the best way to ensure validity of data previously published by others. Papers should be encouraged that replicate studies in the species originally used and in other species. The latter studies reveal whether broad biological principals have been uncovered and increase the chance that similar results would be obtained in man. Where replication fails, the failing authors should be sure their methods are identical to those already published before concluding that the old results are invalid. Reproducibility is the key to the validity of results in studies of animals. Journals should be pleased to publish confirmatory manuscripts.

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Response

Objective assessment of stroke outcome is a difficult task at bedside or on the bench. In a preclinical trial using an animal stroke model, researchers enjoy advantages of a relatively homogenous “animal stroke population,” with similar if not identical demographic factors and uniform stroke pathology. However, substantial variability in stroke outcome from animal to animal still exists in the control group alone. An example is the variability in infarct volume noted in a reproducible focal ischemia model.1 To demonstrate therapeutic efficacy of an intervention without ambiguity, it demands that a treatment effect be detected apart from a number of factors that contribute to variable stroke outcomes. If infarct volume is depicted as an objectively quantifiable end point, the following multiple regression equation can be used to illustrate a multitude of factors that may affect the infarct volume:2

\[ Y_i = T_j + \beta_1 X_{j1} + \beta_2 X_{j2} + \beta_3 X_{j3} + \ldots + \beta_n X_{jn} + e_i \]

where the infarct size \( Y_i \) of the ith animal in the jth treatment group is determined by \( T_j \) (the treatment effect), \( X_1, X_2, X_3, \) and other variables. It is obvious from this equation that treatment effect is only one of a number of factors that determine the infarct volume. An even distribution of variables other than treatment effect between control and treatment groups should be attempted so that a significant difference in infarct volume can be attributed to the treatment under study but nothing else. Physiological parameters (eg, age, gender, metabolic state, blood pressure, body and brain temperature, and arterial blood gases) are but a few of the known variables that can be controlled to minimize their difference between groups. However, there are other factors that are beyond the control of the investigators. The power of randomization exerted by adequate sample sizes will usually (with some exceptions; see References 3 and 4) result in an approximately even distribution of these variables, leaving only the treatment effect to stand out. One of the potential pitfalls in an unblinded study is the introduction of human biases that may cause an uneven distribution of certain factors, including but not limited to those considered by Dr Rosenblum to be “far-fetched.” A sound preclinical trial protocol calls for every effort to evenly distribute variables other than treatment between groups. To what extent the subconscious and conscious biases may affect stroke outcome has not been systematically studied. A conscientious and experienced investigator usually takes steps to avoid “human variables” affecting the outcome in a preclinical trial. Dr Rosenblum proposed an ideal situation: “…at least initially by ignorance on the part of the observer concerning ‘expected’ or hypothesized outcome.” He did not state how to maintain or enforce the investigators’ ignorance.” A convenient and unequivocal means for achieving this goal is to blind all the investigators whose engagement in the trial could conceivably affect the outcome. Blinding of the treatment team does not demand the hiring of additional staff as claimed by Dr Rosenblum, nor was it advocated in the editorial.3 It does
Criteria for valid preclinical trials using animal stroke models.
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