Animal Stroke Models

Clark Millikan, MD

The relevance of animal stroke models to human disease has been addressed in two editorials in Stroke. Wiebers, Adams, and Whisnant raise questions concerning relevance, and Zivin and Grotta respond that animal models are relevant to human disease. From my background of 40 years in clinical and laboratory research in stroke, there are many ideas in both editorials with which I agree and a number of omissions that leave unwritten the seriousness of our national neglect of stroke—from both a laboratory and clinical standpoint.

Neither editorial presents a definition of stroke or points out that the word stroke is a generic one. Stroke is usually defined as an abnormality of blood supply to a focal region of brain. This focal abnormality may be a decrease of nutrients (commonly ischemia) or hemorrhage. The abnormality may vary in duration and quantity, producing an enormous number of transient or permanent neurological defects. The pathogenesis of the focal abnormality may be one or a combination of several mechanisms from a long list that has been published in two classifications of cerebrovascular disease. In a "stroke" patient there may be concurrently congestive heart failure, auricular fibrillation, pulmonary disease, anemia, ulcerated atherosclerotic arterial lesions, and defective collateral flow because of preceding thrombotic lesions. It is obvious that there can never be one model of "stroke" that will mimic all "strokes."

Discussions of animal stroke models seldom deal with this difficulty, and the risk factors associated with human stroke are not simulated in models (problems mentioned by Wiebers et al).

Neither editorial emphasizes that in recent years, most animal models of stroke are models of global or diffuse cerebral ischemia or use highly invasive surgical techniques to occlude the middle cerebral artery. In one extensive review entitled "Rodent Models of Cerebral Techniques," the authors devote less than one-half page of a 16-page article to "miscellaneous models of cerebral embolism and thrombosis," which are the only models they describe that closely mimic human ischemic stroke. These models are criticized because of the variability in number, size, and location of cerebral infarcts. Although this presents difficulty in statistical analysis, this unpredictability is another way these models mimic human stroke. Hopefully, this emphasis on research concerning diffuse cerebral ischemia, as produced by cardiac arrest in the human, will lead to development of protective or healing maneuvers for this important problem. It is no surprise that chemicals appearing to be helpful in "treating" diffuse cerebral ischemia in animals have not worked in human stroke.

Both editorials note, in a single sentence, that very young, healthy animals are used for modeling stroke, whereas 95% of human strokes occur in late middle age or in the geriatric age range and commonly are associated with one or more chronic diseases. This avoidance of the age issue in stroke animal research is amazing—the standard epidemiological principle of age matching is never mentioned in recent extensive reviews of animal models, even though many age-related changes occur in old rats. There is marked decrease in dopamine-stimulated formation of cyclic AMP in old rats compared with that in mature rats, and old rodents have lower dopamine levels than young ones. This may well be analogous to the decrease in the number of dopamine receptors in human brains during aging. Glutamate levels and numbers of glutamate receptors decrease with age in adults. There is a decrease in nerve growth factor immunoreactivity in aged rat brain, and the nerve growth factor level in the hippocampus of 28-month-old rats decreased 40% compared with the level in 6-month-old rats. The disappointing record for identifying clinically effective drugs in animal models of cerebral ischemia may be explained in part by the fact that these trials have all been conducted in young, healthy animals. This is particularly true if the justification of the drug is to alter neurochemistry.

What are the purposes of developing an animal model of stroke? One purpose is to validate hypotheses concerning the pathogenesis of various types of stroke. For example, 35 years ago the notion that an embolus or clot might occlude a vessel, cause an infarct, then break up and "disappear," leaving the vessel open, was not accepted until the model was produced. As simple a hypothesis as "an embolus can go any place the blood goes" is just being understood by some skeptics, only since it has recently been clearly demonstrated in an animal model.

A second purpose for modeling stroke is to discover step by step, at an extracellular and intracellular level, the chemical changes that are caused by decreasing or stopping the supply of blood to the brain. The models commonly used are those of diffuse cerebral ischemia and individual cells in culture or slice preparations, and not focal cerebral ischemia. The former are not stroke models at all but more nearly replicate the situation produced by cardiac arrest in the human, and the latter involve studying cells artificially removed from their normal environment. Years of effort have gone into
unraveling these chemical events, and one editorial cites examples in which beneficial results questionably appeared from a treatment in a model only to fail in humans. Overuse of the "promising" results of pharmacological studies only increases the skepticism concerning the value of "new therapies" coming from "stroke" laboratories. One such article advised testing N-methyl-D-aspartate antagonists (MK801, dextrophan, dextromethorphan, and ketamine) "in patients with brain ischemia." There was no comment about the type, stage, or severity of the strokes to be tested. I believe these studies should continue, but they should at least be performed on old rats and then interpreted with caution by clinicians as well as by nonclinicians.

A third reason for modeling stroke is to produce tissue change that can be used to validate the correlation between an advanced technology such as positron emission tomography, magnetic resonance imaging, or single-photon emission computed tomography and the gross and histological changes seen at sacrifice of the animal. A practical example of potential help to be gained from various stroke models is correlation of the magnetic resonance imaging and computed tomography white matter changes with the underlying pathology in different stroke models. Clinical and pathological correlations have not identified a specific magnetic resonance imaging white matter change characteristic of aging, Alzheimer's dementia, diffuse decrease in cerebral blood flow, or multi-mini infarcts. Imaging of stroke models may help answer some of the questions.

A fourth reason for modeling stroke is to discover the mechanism of an abnormal phenomenon. The pathogenesis of the decreased cerebral perfusion associated with subarachnoid hemorrhage is an example. Years of study and millions of research dollars have not solved the problem. Rather than lamenting the effort, I believe the search must continue.

A fifth reason for modeling stroke is to provide a substrate for testing a potential treatment for both toxicity and efficacy. I reemphasize the warning that there has been unjustified confidence placed in treatment results from inappropriate stroke models that have propagated "numerous further animal and human studies consuming enormous amounts of time and effort to prove that the observation [animal model] has little or no relevance to human disease. . . ." The model and the potential treatment must be matched!!

The five authors express appropriate concern about the mismatch of information from the laboratory to the bedside. The following question appears: "How else can one defend the design of clinical studies that enter patients up to 2 days after stroke onset when no credible animal study has indicated that any drug has neuronal protective properties at that time?" The answer may depend on the pathogenesis and the severity of the stroke. What is the severity of the neurological defect at 48 hours after onset? In some patients the deficit has disappeared, while other patients are dying with progressing cerebral edema. Each author is fully clinically acquainted with the tremendous variation in the temporal profile of stroke in humans. And yet this readily available information is ignored in both animal and human studies of "treatment." How has this obvious mismatch of information from the laboratory and from the bedside occurred? Zivin and Grotta write, "The clinical investigator must reconcile laboratory results with clinical reality, make an unbiased assessment of a drug's applicability to human stroke patients..." The results of experimental data on "stroke" in young rats are not necessary for the experienced clinician to know that a patient who has a flaccid hemiplegia and decreased consciousness 36 hours after onset is damaged forever. The writers identify that "communication among researchers is also a problem." It is the responsibility of the clinical investigator, with or without experience in the laboratory, to recognize that MK801 is not going to regrow infarcted neurons several hours after stroke onset regardless of the "promising" results obtained from models of diffuse cerebral ischemia. Pulsinelli and Buchan mention "inexperience and scientific frailties of the investigator," "extreme pressure to publish first," "less-than-ideal critical objectivity on the part of the investigator," and "weak experimental design and technique as some of the reasons there is" failure of the pharmacological studies to translate from the animal to the clinical setting. Another important factor in this situation is that many laboratory investigators never see a stroke patient. There is no question about the need for a forum that brings together clinical and basic scientists. For 2½ decades there was such a forum (Princeton Conferences); the content of the conferences has gradually changed so that clinical presentations are limited to about 15% of the program.

What are some of the problems in stroke research that have generated these two editorials? Failure to define stroke is a major reason for confusion and for the wasting of "enormous amounts of time and effort." Each of the five authors of the two editorials is fully clinically acquainted with the tremendous variation in the temporal profile of stroke in humans. The basic scientist who does not see patients is not acquainted with the extraordinary number of variables that make human stroke such a complex problem and that many models may be necessary to begin to get answers to what may appear to be simple questions. Even when a specific mechanism is involved (i.e., an embolus to a brain vessel) and there are no associated risk factors, there are numerous important variables, including the type of the embolus, the size of the embolus, the duration of the occlusion, and the immediate availability of collateral perfusion. There is great need for clinical investigators who can display and explain these clinical problems to investigators without clinical training.

A fundamental problem that has influenced the sluggish advance of research concerning stroke (focal cerebrovascular disease) is the quantity and source of support for both clinical and basic research. Compared with cardiovascular disease, cancer, and mental illness, stroke receives only token amounts of money from the National Institutes of Health. Two alternative sources of funding, to which investigators are frequently turning, are the pharmaceutical industry and equipment manufacturers. One editorial, in discussing a compound (MK801), points out that these sources of funds may generate many studies and reports that have little relevance to human stroke. The other editorial deals with a part of this problem as Zivin and Grotta write "... or are motivated by considerations related to drug marketing or completing a study as rapidly as possible in the largest possible number of subjects."
Neither editorial notes the poverty of activity concerning stroke in many of our medical schools, schools that are the foci for teaching and research about heart disease and cancer. Less than half of US medical schools have any major emphasis on stroke. I hope that the concept "the decade of the brain" will include appropriate attention to the nation’s No. 1 neglected killer.

References
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Animal stroke models.
C Millikan

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