On Ischemic Brain Injury in Genetically Altered Mice

In recent years, genetically altered mice, either overexpressing or deficient in specific gene products, have come to play a vital role in experimental studies designed to probe the molecular pathophysiology of ischemic brain injury. More than 100 such studies have been reported to date, and notable successes have been achieved in elucidating the roles of the neuronal, endothelial, and inducible isoforms of nitric oxide synthase and of the cytosolic (CuZn) superoxide dismutase in brain ischemia. Other studies of cerebral ischemia have used mutant mice to assess altered glutamate-receptor subunit composition, vascular adhesion molecules, gene products related to nuclear damage, cell death and survival, apoptosis, transcription factors and early-response genes, cytokines, and apolipoprotein E. However, interpretation of these results is fraught with pitfalls, however, and this is particularly the case in studies comparing the responses of wild-type and mutant mice, in which it becomes essential that mice of all groups be bred on a similar genetic background. The importance of rigorous control of genetic background in mutant mouse studies has been emphasized repeatedly in recent publications. Elsewhere in this issue, Tabrizi and coworkers confront this problem in a careful study designed to assess whether endogenous tissue plasminogen activator (tPA), in fact, is beneficial or detrimental in focal cerebral ischemia. An impetus to their study is the recent report of Wang et al, which purported to demonstrate that mice deficient in tPA exhibited 50% smaller cerebral infarcts than did wild-type mice. As noted by Tabrizi et al, however, interpretation of that study was complicated by the fact that it compared C57BL/6 wild-type mice against C57BL/6 tPA-deficient animals. In the present study, Tabrizi et al show that C57BL/6 wild-type mice develop almost 10-fold larger infarcts than do mixed 129/Sv--/-- and C57BL/6--/-- animals. In the recent study of Nagai et al, who used an MCA permanent-ligation model to demonstrate that inactivation of the tPA gene led to significantly reduced infarct size compared with that in wild-type mice. These workers, it should be noted, reported a 45% larger mean infarct volume in wild-type C57BL/6 mice than in S129 mice and an intermediate infarct size in mice with 50%/50% mixed wild-type backgrounds. However, they did not specify the genetic background of their tPA-knockout animals, and they did not state that endogenous tPA deficiency in fact exacerbates the consequences of focal cerebrovascular occlusion by encouraging vascular fibrin deposition, enhancing ischemia-induced brain blood-flow decrements, and increasing the volume of the resulting brain infarct compared with wild-type animals. These results tend to vindicate tPA by alleviating the potential concerns raised by the earlier study and by demonstrating that endogenous tPA likely protects the ischemic brain by promoting thrombolysis. Nonetheless, points of confusion remain, in that Tabrizi et al do not precisely explain what they mean by a “matched mixed 129/Sv and C57BL/6 genetic background” in their study. Were their tPA--/-- and tPA+/- animals, in fact, genetically identical except for the mutant allele? Or was there animal-to-animal variation in the extent of contribution of the genes from each genome? These issues deserve clarification.

Of interest, a recent experimental study designed to evaluate the putative neurotoxicity of exogenous tPA in standardized rat models of both global and focal cerebral ischemia has also convincingly shown that tPA administered at clinically relevant doses fails to exacerbate ischemic injury in either setting. These studies exonerating tPA have considerable practical import, in that recombinant tPA (rtPA) administered to patients within 3 hours of the onset of an ischemic stroke has recently been shown in an exemplary multicenter trial to improve neurological and functional outcome significantly, and this pharmacological agent is now in routine clinical use as a therapy for acute ischemic stroke.

The report of Tabrizi et al also highlights a number of other issues crucial to the proper experimental study of brain ischemia. Precise monitoring and regulation of multiple physiological variables—notably blood pressure, arterial oxygenation, and pH, and systemic as well as brain temperatures—is essential to avoid the confounding influence of physiological variations (eg, brain temperature) on the outcome of an ischemic insult. Consistency of experimental technique is also crucial in ensuring reproducible infarct volume in intraluminal filament occlusion models of focal cerebral ischemia. We have shown in the rat that coating the filament with poly-L-lysine markedly enhances the reproducibility and consistency of the resulting cerebral infarct, presumably by strengthening the adherence of the occluding filament to the adjacent endothelial surface; and we have extended this method to the mouse. The concerns expressed above can be raised in respect to the recent study of Nagai et al, who used an MCA permanent-ligation model to demonstrate that inactivation of the tPA gene led to significantly reduced infarct size compared with that in wild-type mice. These workers, it should be noted, reported a 45% larger mean infarct volume in wild-type C57BL/6 mice than in S129 mice and an intermediate infarct size in mice with 50%/50% mixed wild-type backgrounds. However, they did not specify the genetic background of their tPA-knockout animals, and they did not...
appear to monitor or control physiological variables (apart from body temperature).

In experimental models of focal cerebral ischemia, apparently “minor” differences in the extent of decline of local cerebral blood flow (CBF) may result, in fact, in major differences in infarct volume. By using pixel-based autoradiographic and histopathological image-mapping strategies, our group has shown that the precise extent of local CBF decrement during a 2-hour period of MCA occlusion exquisitely determines the probability of histopathological infarction at the same site 3 days later. Thus, regions having flow in the “ischemic core” range (ie, CBF 0% to 20% of control values) have a 96% probability of undergoing infarction. By contrast, tissue zones with only slightly higher ischemic CBF levels (30% to 40% of control) have only an 80% probability of infarction, and zones of still higher CBF are largely spared from infarction. In the study reported in this issue, Tabrizi et al observed an important difference in relative CBF measured in their tPA−/+ versus tPA−/− mice, both during and after MCA occlusion. CBF measured at corresponding sites declined during ischemia to 20% of control values in tPA-deficient mice but remained at 30% of control in wild-type animals; the former group also showed less-complete postischemic return of CBF than the latter. Differences of this magnitude are fully sufficient, of themselves, to account for the effect of tPA deficiency in enlarging the volume of cerebral infarction.

The determinants of CBF during and after brain ischemia are multifactorial; thus, at least some genetic alterations influence the outcome of an ischemic insult by directly affecting the extent and/or distribution of the CBF decrement produced by the ischemic insult (eg, see Reference 3). This appears to be the case in the study of Tabrizi et al. Interstrain differences in intracranial vascular anatomy affecting the circle of Willis are another factor influencing the degree of ischemia. As the methods of molecular biology and genetics continue to be applied with increasing success to the study of ischemic brain injury, one expects not only an increasingly sophisticated understanding of pathophysiology but also major advances in the rational design and implementation of neuroprotective strategies that will be tested in the clinic and succeed, eventually, in diminishing death and disability in patients with ischemic stroke.

Myron D. Ginsberg, MD
Peritz Scheinberg Professor of Neurology
Director, Cerebral Vascular Disease Research Center
University of Miami School of Medicine
Miami, Fla

References


On Ischemic Brain Injury in Genetically Altered Mice
Myron D. Ginsberg

Arterioscler Thromb Vasc Biol. 1999;19:2581-2583
doi: 10.1161/01.ATV.19.11.2581
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/11/2581

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/