Cerebral ischemia in heme oxygenase-2-deficient mice: the second filament makes the difference

Holger Scholz and Nicole Wagner
Johannes-Müller-Institut für Physiologie, Medizinische Fakultät Charité, Humboldt-Universität Berlin, 10117 Berlin, Germany

SINCE THE ADVENT of modern embryonic stem cell technology, mice with targeted gene deletion have come into use as model organisms to study the mechanisms of protection from ischemic injury. Heme oxygenases (HO) have gained considerable interest because of their potential neuroprotective effect (1, 8). HO converts pro-oxidant heme to biliverdin and carbon monoxide (CO), which may act as a neurotransmitter, vasodilator, and anti-apoptotic factor (11, 13). Biliverdin is rapidly transformed to bilirubin, which can protect neurons from oxidative stress at low concentrations (4).

Transgenic overexpression of heme oxygenase-1 (HO1) under control of a neural promoter markedly reduced neuronal cell damage in response to occlusion of the middle cerebral artery (MCA; 10). On the other hand, infarct volume after transient MCA occlusion (MCAO) was significantly enlarged in mice with homozygous disruption of the gene encoding heme oxygenase-2 (HO2) compared with wild-type (WT) animals (3). These reports support the concept that endogenous HO activity may exert a neuroprotective effect during focal cerebral ischemia.

Variations in vascular anatomy between knockout and WT mouse strains can impose a major drawback on studies performed to assess the effect of a single gene defect on cerebral ischemic injury. In particular, it has to be considered that differences in regional blood flow due to anatomic variations of the cerebral vasculature may account for variable infarct volumes in WT and mutant mice (6, 7, 9, 14). In this issue of the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, Goto and colleagues (5) describe a novel technique of transient MCAO that produces consistent and reproducible cerebral infarcts in a knockout strain of mice in which the standard procedure was ineffective. The authors make use of their new model to demonstrate that the greater infarct volume in HO2-deficient (HO2−/−) mice is likely due to the lack of a neuroprotective effect of HO2 activity rather than reflecting differences in cerebral blood flow between WT and mutant mouse strains.

Why is this novel technique advantageous compared with standard procedures? Focal cerebral ischemia can normally be produced by lowering blood flow to the brain through intraluminal insertion of a single nylon filament into the internal carotid artery (12). Although this standard monofilament technique caused large and consistent infarcts in many mouse strains, its use in knockout animals turned out to be frequently ineffective due to variations in cerebral vascular anatomy (6, 7). To overcome these difficulties, Goto et al. reduced the blood flow into the MCA territory from both the anterior and posterior portion of the circle of Willis. For this purpose, they inserted two 6-0 nylon filaments into the left internal carotid artery, 6.0 and 4.5 mm past the pterygopalatine artery junction, in a way that the blunted tips of the two monofilaments lay distal and proximal to the origin of the MCA. After 1 h of MCAO, the infarct volume was significantly greater in HO2−/− mice compared with WT animals (44 ± 6 vs. 25 ± 3%). In contrast, the tissue volume at risk of ischemic injury, which was determined by brief perfusion with a 2% Evans blue solution, was similar in WT (59 ± 2% of hemisphere) and HO2−/− mice (62 ± 4%). Also the volume of tissue with cerebral blood flow <50 ml·min⁻¹·100 g⁻¹ was comparable in WT (35 ± 9%) and mutant mice (36 ± 11%) during MCAO and at 3 h of reperfusion. The infarct volume was significantly increased in WT but not in HO2−/− mice by infusion of the HO inhibitor protoporphyrin IX (SnPPIX) during reperfusion after 1 h of MCAO. The lack of effect of SnPPIX on total infarct volume in HO2−/− mice suggests that this compound acted primarily through inhibition of HO2 rather than HO1 activity. One can therefore assume that neuroprotection against ischemic injury is mainly provided by the neuronally constitutive HO2 isoform. Furthermore, because SnPPIX enhanced the lesions when applied only during the reperfusion period, it seems that HO2-related protection of neurons normally becomes effective after the ischemic insult. As a major strength, the paper by Goto et al. convincingly demonstrates that the larger infarct volume in HO2−/− mice is not attributable to a greater volume at risk, lower intras ischemic blood flow, or poor reflow compared with WT animals, but rather to a direct neuroprotective effect of HO2 activity. It remains to be determined whether neuroprotection by HO2 in response to ischemia is related to inhibition of apoptotic cell death or removal of pro-oxidant heme (2). In either case, the new double-filament model of focal cerebral ischemia may be of important value for future studies that are intended to analyze the molecular mechanisms of neuron protection in murine knockout strains.
REFERENCES


