SUPPLEMENTAL MATERIALS

Criterion for defining microinfarcts and microhemorrhages in rodent studies.

Recent reviews have established guidelines for identifying human cerebral microinfarcts[1] and microhemorrhages.[2] However, no criteria yet exist for defining spontaneously occurring microlesions in rodent models. Microinfarct size is the primary means to distinguish these lesions from larger ischemic strokes caused by blockage or rupture of major cerebral arterioles. We suggest that an upper size threshold of 1 mm lesion diameter be used for microinfarcts. This would encompass most microinfarcts observed across the preclinical studies reviewed here, and is also consistent with the size of penetrating arteriole perfusion domains observed in rats and mice. A caveat, however, is that microinfarct shapes can be irregular and individual tissue slices may not transect the widest region of the lesion. It is therefore best to report microinfarct volume, if possible, by measuring lesion area over multiple adjacent brain slices. In addition to defining a region of infarction with loss of NeuN or Hematoxylin & Eosin pallor, it is recommended to also confirm the presence of neuroinflammation by staining for CD68, Iba1 or GFAP, which should be upregulated following tissue ischemia. For old microhemorrhages, stains such as Perl's Prussian blue should be used to detect the presence of iron. We suggest that lesions should consist of at least 5 iron-positive depositions as a lower threshold and the overall region of staining be less than approximately 0.5 mm in diameter. We noticed that regions with only a single iron deposit were counted as microhemorrhages in some studies, and these may be too small to represent true microhemorrhages. By standardizing the reporting of microinfarcts and microhemorrhages with lesion size criterion, and data on lesion size range, brain location, and prevalence, consistency among research groups and comparisons between different models can be evaluated more rigorously.

References

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2. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, et al. Cerebral microbleeds: A guide to detection and interpretation. *Lancet Neurology*. 2009;8:165-174