Reperfusion-Based Hemorrhagic Transformation in Spontaneously Hypertensive Rats: A Model for Translational Research

E. C. Henning[1], L. L. Latour[1], J. M. Hallenbeck[1], and S. Warach[1]
[1]National Institute of Neurological Disorders and Stroke, Bethesda, MD, United States

Introduction

The risk of hemorrhagic transformation (HT) in acute stroke patients is exacerbated by reperfusion injury following administration of intravenous rtPA. Recent reports both clinically[1,2] and experimentally[3-5] suggest a relationship between blood-brain barrier (BBB) disruption and the incidence and severity of HT. Although experimental animal models of stroke have attempted to predict HT, many have focused on rtPA-induced HT and BBB breakdown[3-6]. Others have investigated reperfusion-based HT and BBB breakdown, but only in young, healthy rats[7-8]. In order to evaluate the effect of reperfusion on HT with and without rtPA, experimental models more closely associated with patient pathophysiology (hypertension, increased risk of HT) are necessary. For this reason, we introduce a model of reperfusion-based HT in SHR rats.

Methods

Ten spontaneously hypertensive (SHR) rats weighing 300-360g were subjected to 30 minutes of MCA occlusion using the suture model. Gradient-echo (GRE) imaging was performed using a Bruker Biospin 7.0T/30cm imaging spectrometer on days 1, 2, 3, 4, and 7 for longitudinal evaluation of HT. In a subset of animals (n = 5) following imaging on day 7, serial histology using hematoxylin-eosin (H&E) staining was compared with the correlative MRI imaging slices for visual assessment and scoring of HT. Hemorrhage volume measurements (including long axis diameter (LAD)) and semi-quantitative scoring were performed by a blinded observer. Values are expressed as mean (SD). Two-tailed t-tests assuming unequal variances were performed to assess severity of hemorrhage on day 7 post-MCAO.

Results

HT was evident on GRE images in 5 of 10 animals by day 1 (1.32 mm^3 (1.35), range = 0.37-3.05 mm^3), and in 10 of 10 animals by day 2 (1.89mm^3 (3.21), range = 0.07-10.85 mm^3). HT volume continued to increase out to day 7 (8.03mm^3 (10.22), range 2.18-38.07 mm^3). All animals exhibited evidence of punctate (score 1, LAD = 113μm (4)) and confluent petechial (score 2, LAD = 421μm (261)) hemorrhage. Four animals had macroscopic, non-space-occupying hemorrhages (score 3, LAD = 0.980 (0.597)) and one exhibited a macroscopic space-occupying hemorrhage (score 4, LAD = 2.053 (0.486), Fig.1).

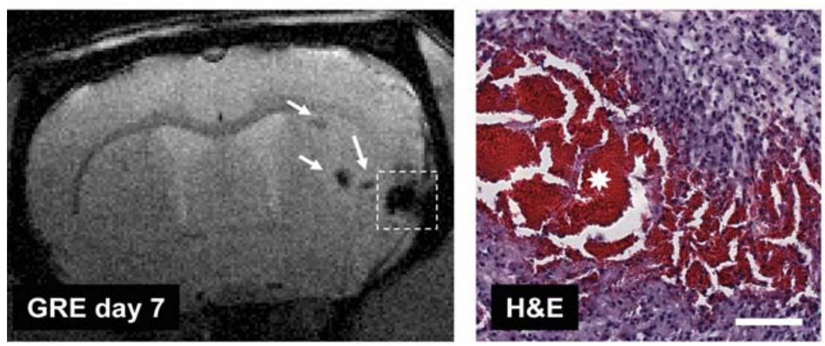

Figure 1. Hemorrhagic transformation (HT) for a representative animal on day 7 post-reperfusion. Left: GRE image (TR/TE = 350/12 ms). Hypointense regions represent HT (arrows, square ROI). Right: Corresponding H&E stained section (20×). This animal exhibited a large space-occupying hemorrhage (star), score 4. Scale bar = 500μm.

Conclusions

This model has a high-incidence of macroscopic hemorrhages (non-space-occupying and space-occupying) and should be useful for the evaluation of reperfusion-based HT with and without rtPA, as well as in the development of future stroke therapies and their effect on BBB integrity.

References

[1] Latour LL et al. Ann Neurol 2004;56(4):468-477.
[2] Warach S, Latour LL. Stroke 2004;35[suppl 1]:2659-2661.
[3] Dijkhuizen RM et al. J Cereb Blood Flow Metab 2001;21(8):964-971.
[4] Dijkhuizen RM et al. Stroke 2002;33(8):2100-2104.
[5] Neumann-Haefelin C et al. Stroke 2002;33(5):1392-1398.
[6] Brinker G et al. Neuroreport 1999;10(9):1943-1946.
[7] Neumann-Haefelin T et al. Neuroreport 2001;12(2):309-311.
[8] Neumann-Haefelin T et al. Stroke 2000;31(8):1965-1972.