Clots and Blots: A Stroke of Good Fortune in Identifying Protein Changes in Cerebral Accidents

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doi: 10.1111/j.1755-5949.2011.00268.x

The important study “Increased oxidative stress is responsible for severer cerebral infarction in stroke-prone spontaneously hypertensive rats” by Ding-Feng Su and his colleagues in Shanghai [1] marks a new approach to determining intracellular signaling and gene regulation of vascular function in stroke. Although the study focused on an animal model of stroke that is due to a combination of hemorrhagic and ischemic events, the method can easily be applied to other vascular complications in the brain and elsewhere. While the study does not provide any clear answers, it does however point to a new direction for others to consider in their studies of several conditions that may involve changes in gene regulation, e.g., dementia, appetite regulation, cerebral ataxia, cerebral and peripheral aneurysms, vascular remodeling, vascular complications in diabetes and so on [2,3]. Thus, the paper by the group led by Su describes a methodological approach that lends itself well to a number of experimental paradigms.

It is somewhat appropriate that this article originates in China, since recent studies confirm that the incidence of stroke in China is more than five times that of myocardial infarction and as importantly, intracerebral hemorrhage is responsible for about one-third of all strokes in China, which is nearly three times the frequency in North America and elsewhere [4]. An added dimension is that there is a greater (three times) incidence of stroke in the northern parts of China than in the south [5]. Thus, it is not surprising in that there are more patients with stroke in China than elsewhere in the world, and stroke represents the second most important cause of death and morbidity in China (with cancer and respiratory diseases being the leading causes) [5]. Stroke in developing countries accounts for ~70% of the world death rate from stroke, and 40% of these stroke related deaths in developing countries are in China alone [6].

The incidence of hemorrhagic strokes and its associated mortality is positively related to the level of poverty: with increased economic development in China, there has been a small (~2%) reduction in hemorrhagic stroke but a significantly greater (~9%) incidence of ischemic strokes [7]. A fortunate consequence of this growing economic strength in China is the increased capacity for leading edge biomedical research, as shown in the paper by Su

and his colleagues. This epidemiological transition in the incidence of stroke incidence is largely driven by economic influences on lifestyle choices. This means that it is important that better insight is gained into understanding stroke prevention and also improved management strategies.

The paper by Zhang et al. uses a protein expression profile in a rat model of spontaneously hypertensive rats who are stroke prone (SHR-SP) [1]. Some of these animals were treated with a combination of vitamins C and E. The protein expression profiles related to oxidative stress were then compared in SHR and SHR-SP that were either untreated or treated with antioxidants. The SHR-SP is a frequently used rodent model of spontaneously developing strokes that are triggered by overt hypertension. The premise of the study by Zhang et al. is that by using two genetically related animal models (SHR and SHR-SP) that do not differ in their blood pressures, then differences in protein expression in the SHR-SP model will be unique to that strain and unrelated to the blood pressure (specific changes that are driven by blood pressure are assumed to be equivalent in the two strains). The group led by Ding-Feng Su in Shanghai reasoned that a global screen of differential expression in SHR-SP versus SHR would unveil changes in protein expression that were derived in an unbiased manner— that is, with no preconceived notions of proteins of interest. This would then allow novel mechanisms to be uncovered. They used a two-dimensional (2D) fluorescence gel electrophoresis method, which allows resolution of a large number of proteins on a single gel. The data suggested a loss of antioxidative capacity in SHR-SP that was ameliorated by the supplementation of exogenous antioxidants.

The method they used started with the homogenization of whole brains (SHR-SP brains were shown to have a larger area of cerebral infarcts) and upon extraction of protein samples, they were labeled with a fluorophore before separation on 2D gel electrophoresis. The 2D gels were then scanned for the fluorescently labeled proteins at a 100 μ m resolution and the densities for various proteins compared in gels from SHR-SP and SHR brains to determine differential expression of all proteins. Protein spots were subsequently analyzed using mass spectroscopy and 2D-Western blot methods and peptides identified through a search of protein

sequence databases. Using these methods, the authors detected nearly 2000 spots on the 2D gels, most of which were present in gels from both SHR-SP and SHR brains. But of interest is that 25 proteins that were specifically downregulated in SHR-SP while another 18 protein spots were upregulated in the brains from SHR-SP. Further analysis by peptide mass fingerprinting and mass spectroscopy allowed for the categorization of the downregulated proteins into four broad classes: oxidative-stress related proteins, glucose metabolism related proteins, signal transduction related proteins and a "miscellaneous" category. Thus proteins found to be downregulated in SHR-SP included a 1.3-fold change in glutathione transferase, with added reductions in total antioxidant capacity and glutathione peroxidase as indicators of an increased oxidative stress in SHR-SP. Treatment of SHR-SP with antioxidants such as vitamins C and E were able to reduce markers of oxidative stress in SHR-SP. Since changes in protein expression were specific to the SHR-SP, the authors were able to implicate stroke induced alterations that were independent of those produced by hypertension alone.

Oxidative stress is involved in a number of clinical pathologies and also in normal physiology and development; the role of oxidative stress in cerebrovascular diseases is well recognized [8,9]. The levels of oxidative stress reflect a balance of free radical generation and free radical removal capacities. Thus, it is unclear from the study by Zhang et al. if there were also increases in free radical (reactive oxygen and nitrogen) species, and also which specific isoforms of the superoxide generating systems could have been upregulated in SHR-SP [10]. An extension of these findings would be to also determine if oxidized hemoglobin is able to downregulate antioxidant capacity and increase free radical generation—for example in a cultured cerebral artery preparation. Others have already shown that lysed blood can induce gene expression for stress proteins and also cause DNA fragmentation in rat brains [11]. While it is of interest that treatment with vitamins C and E was beneficial in reducing infarct size etc. in SHR-SP, the clinical

relevance of this in a patient setting is unclear as there is little evidence to suggest that antioxidants may be able to provide relief in cerebral accidents.

In the paper by Zhang et al., the Western blot study compared brain tissue from SHR versus SHR-SP and determined which changes in protein expression in the latter by a "ratio metric method," akin to for example ratio metric data for some fluorescent probes for intracellular calcium etc. One difficulty with this method is to determine where to set the appropriate "threshold" in this ratio of expression of various proteins the two strains of SHR—so how much of an increase/decrease in this ration is needed to identify useful targets be probed? Another aspect related to this is that it is quite likely that there may be changes in cerebrovascular function, which may be unrelated to increased expression of specific proteins, e.g., due to altered activity of, for example, Rho-kinase [12].

A related study from Andriantsitohaina's group in France also examined differential expression of proteins following middle cerebral artery occlusion (for 90 min) in normotensive rats; 5 proteins were upregulated while another 14 proteins were downregulated by cerebral ischemia in untreated rats [13]. These authors reported that even a relatively short period of cerebral ischemia caused differential expression of a number of proteins that were subject to oxidative and nitrosative stress—importantly, pretreatment of rats with red wine polyphenols, which have antioxidant properties, halved the number of proteins that were downregulated by cerebral ischemia [13]. The study by Zhang et al. [1] provides strong evidence that cerebral infarct area in SHR-SP is caused by increased oxidative stress—which in turn is related to specific changes in antioxidant profile that are unrelated to hypertension. This increased oxidative stress exacerbates the extent of cerebral infarcts in SHR-SP and suggests that a targeted approach to normalize markers of oxidative stress could be a useful strategy in managing stroke and its untoward neurological impact.

References

- Zhang X-H, Lei H, Liu A-J, Zou Y-X, Shen F-M, Su D-F. Increased oxidative stress is responsible for severer cerebral infarction in stroke-prone spontaneously hypertensive rats. *CNS Neurosci Ther* 2011;**17**:590–598.
- Cecchetti A, Rocchiccioli S, Boccardi C, Citti L. Vascular smooth-muscle-cell activation: Proteomics point of view. *Int Rev Cell Mol Biol* 2011;**288**:43–99.
- Rocchiccioli S, Citti L, Boccardi C, et al. A gel-free approach in vascular smooth muscle cell proteome: Perspectives for a better insight into activation. *Proteome Sci* 2010;**8**:15.
- Jiang B, Wang WZ, Chen H, et al. Incidence and trends of stroke and its subtypes in China: Results from three large cities. *Stroke* 2006;**37**:63–68.
- Liu M, Wu B, Wang WZ, Lee LM, Zhang SH, Kong LZ. Stroke in China: Epidemiology, prevention, and management strategies. *Lancet Neurol* 2007;**6**:456–464.
- Silva G, Jeney V, Chora A, Larsen R, Balla J, Soares MP. Oxidized hemoglobin is an endogenous proinflammatory agonist that targets vascular endothelial cells. *J Biol Chem* 2009;**284**:29582–29595.
- Zhao D, Liu J, Wang W, et al. Epidemiological transition of stroke in China: Twenty-one-year observational study from the Sino-MONICA-Beijing project. *Stroke* 2008;**39**:1668–1674.
- Chrissobolis S, Miller AA, Drummond GR, Kemp-Harper BK, Sobey CG. Oxidative stress and endothelial dysfunction in cerebrovascular disease. *Front Biosci* 2011;**16**:1733–1745.
- Miller AA, Budzyn K, Sobey CG. Vascular dysfunction in cerebrovascular disease: Mechanisms and therapeutic intervention. *Clin Sci (Lond)* 2010;**119**:1–17.
- Touyz RM, Briones AM, Sedek M, Burger D, Montezano AC. NOX isoforms and reactive oxygen species in vascular health. *Mol Interv* 2011;**11**:27–35.
- Matz P, Weinstein P, States B, Honkaniemi J, Sharp FR. Subarachnoid injections of lysed blood induce the hsp70 stress gene and produce DNA fragmentation in focal areas of the rat brain. *Stroke* 1996;**27**:504–12; discussion 513.
- Shimokawa H, Takeshita A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler Thromb Vasc Biol* 2005;**25**(9):1767–1775.
- Ritz MF, Ratajczak P, Curin Y, et al. Chronic treatment with red wine polyphenol compounds mediates neuroprotection in a rat model of ischemic cerebral stroke. *J Nutr* 2008;**138**:519–525.