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Microtubule Associated Proteins (MAP) and Motor Molecules: Direct Tissue MALDI Identification and Imaging

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ABSTRACT

Goal: The effect of cerebral ischemia on MAP proteins, microtubules and motor molecule kinesin in the biochemical cascade of acute stroke.

Background and Significance: Stroke is the third cause of death in the United States and the direct or indirect medical expense is in excess of 50 billion dollars per annum. Only 3% of stroke victims are eligible for thrombolytic therapy. Apoptosis and necroptosis in acute stroke extend the focal anatomic injury, retard recovery, and increase mortality. Cerebral ischemia immediately interdicts ATP production which effects MAP proteins, microtubule integrity, and motor molecule processivity. This process brings cellular metabolism to an abrupt halt. Understanding the acute biochemical cascade should allow novel therapeutic intervention.

Methods: A validated non-invasive acute stroke murine model developed by the authors was used to identify β tubulin by direct tissue MALDI in a previous report. In this study coronal stroke brain tissue sections were obtained at timed intervals during and following stroke induction. Mirror image sections were subjected to MALDI imaging and direct tissue protein identification. Adjacent sections were used for high-pressure protein extraction (Barocycler, Pressure Biosciences, West Bridgewater, MA), and fluorescent microscopic imaging of the tubules.

Results: Post stroke identification of kinesin changes in MAP 1, tubulin and, and the microtubules were identified in progressive fashion during stroke induction and the post stroke 60 minute interval. These changes were correlated with progressive dissolution of the microtubules. They were not seen in the non-stroke contralateral hemisphere (control).

Conclusion: This pilot study demonstrates the relationship between acute brain ischemia, clinical stroke, kinesin, MAP 1 proteins, and dissolution of the microtubules. The specific aim of the study was to use Imaging MALDI as an adjunct to MALDI in understanding the chemistry of clinical stroke.

1. Stroke model- A validated murine stroke model developed by the authors was used to initiate the biochemical cascade of acute stroke.{{3 Pevsner,P.H. 2001;220 Eichenbaum,J.W. 2002; }} The study was approved by the NYU Institutional Animal Care and Use Committee (IACUC) Protocol # 050409-3. Rose Bengal, a Xanthine dye, is injected percutaneously into the abdomen (peritoneal cavity) of a mouse. The scalp is irradiated with visible light through a 3mm fibreoptic light-pipe applied to the murine scalp over the parietal lobe of the murine brain. There is a photochemical reaction (beneath the light pipe tip) between the circulating Rose Bengal in the underlying brain and the irradiated light yielding singlet oxygen. Singlet oxygen, a reactive oxygen specie, with indigenous nitric oxide initiates lipid peroxidation in the cell membranes of brain cells (neurons and glia). This leads to metabolic interruption of cellular respiration and adenosine triphosphate and guanidine phosphate production, early identification of a kinesin motor molecule isoforms which "fall off " the microtubule, loss of microtubule associated protein integrity, and finally microtubule dissolution with release of ß-tubulin, and initiation of apoptosis, programmed cell death. This outline of the biochemical cascade is a shorthand incomplete description of process. Future therapy of human stroke may target this pathway. {{1379 Jameson,L. 1980;1380 Halpain,S. 1990; 1384 Hoshi,M. 1992; 1438 Fygenson,D.K. 1994; 1284 Pettigrew,L.C. 1996; 1372 Desai,A. 1997; 1387 Llansola,M. 2001; 1435 Fygenson,D.K. 2004; 1285 Krebs,A. 2005; 1378 Moores,C.A. 2006; 1290 Snyder,J.P. 2007; }}

2. Tissue Imaging MALDI is a new tool for normal and pathologic tissue examination with unique sample preparation requirements for fixation and matrix application. Meticulous attention to the usual principles of tissue preservation are critical. Destructive changes of autolysis (tissue putrefaction) freezing artefact, and chemical corrosion must be avoided. by adequate fixation without cross linking tissue proteins. Imaging MALDI should be prepared with the same care.

   a. Autolysis (tissue putrefaction)
      i. Definition- Autodigestion of tissue by indigenous proteolytic enzymes.
      ii. Description- Loss of cell membrane and intracellular integrity (tissue putrefaction).
      iii. Prevention- Fixation by perfusion and/or immersion of living tissue or biopsy specimens in ethyl or methyl alcohol without tissue or cellular protein cross linkage.
   b. Freezing Artifacts
      i. Definition- Mechanical destruction of tissue and cellular integrity by ice crystals. Figure 1.
      ii. Prevention- Cryoprotection of cells and tissue by perfusion and/or immersion with DMSO 2%, Glycerol 20%, and ethyl or methyl alcohol 78%.
   c. Chemical Artifacts
      i. Definition- Destruction of tissue and cellular integrity by chemical corrosion, e.g., acetonitrile (ACN), and trifluoracetic acid (TFA). Figure 2.
      ii. Prevention- Substitue ethyl or methyl alcohol for ACN/TFA matrix (CHCA, Sinapic Acid, or DHB) solvent.
   d. Matrix Application
      i. Use recrystallized matrix of the highest purity dissolved completely in ethyl or methyl alcohol. If the mixture is a solution and not a colloid the matrix crystals will be finer and more compact af ter the alcohol vaporizes. In our experience there is little difference in matrix application using mist, chemical printer, atomizer or micropipette. Figure 3.

Experimental

Timed stroke images and murine neurological inventory- Stroke induction was performed with 5 minute, 8 minute, and 10 minute light irradiation. A murine neurologic physical inventory was correlated with the tissue Imaging MALDI, gross brain surface and histopathology. Terminal percutaneous transthoracic cardiac perfusion with an aqueous 1 mL solution of DMSO 2%/Glycerol 20% was performed at 60 minutes post stroke induction.{{222 Eichenbaum K., Eichenbaum J., Fadiel A., Miller DC., Demir N., Naftolin F., Stern A.,and Pevsner P. 2005 2005; }} The brain was rapidly removed, bathed in an aqueous solution of DMSO 2%/Glycerol 20%/ ethyl alcohol 78%, and immediately immersed in liquid nitrogen cooled semi-solid isopentane.

Contiguous one micron cryosections were cut from the cryoprotected frozen brain and used for histopathology, Figure 4. and Imaging MALDI, Figure 5.

A small piece of stroke tissue from another 10 minute induced stroke brain was processed with a high pressure protein extraction (Pressure Biosciences, Barocycler). Figure 6. The extracted protein in PBS buffer was filtered with a 3000 Da micropore filter, and the filtrate trypsinized, Porcine trypsin 1ug/mL v/v with 1 uL of sample. The sample was analyzed by MALDI with sinapinic matrix 10mg/mL in 50/50 vol/vol ACN/0.1% TFA. NCBInr data base analysis and Mascot software were used for PMF identification of two kinesin isoforms proteins. Figure 7.

Conclusion

This study provides a paradigm for avoiding freezer artefact and chemical corrosion, and furnishes ultrathin, matrix coated tissue sections for Imaging MALDI. Imaging MALDI can be used as a correlative imaging modality with histopathology.We utilized high pressure to extract protein from tissue in simple buffer without further chemical exposure of the tissue sample. Two kinesin isoforms (motor molecules) representing very early change in stroke tissue were identified with MALDI. These early chemical changes strongly correlate with the clinical findings of neurologic deficit in the murine stroke model.

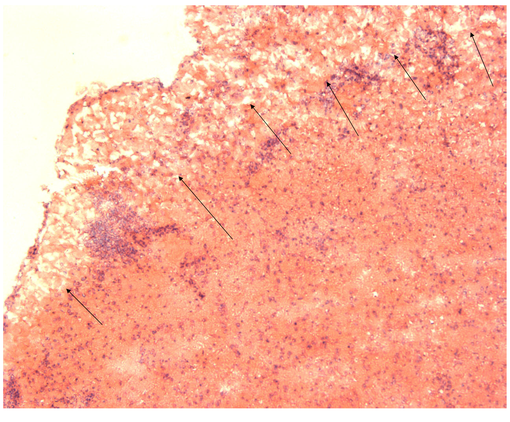

Figure 1. Freezer artifact (clear spaces) in tissue section of stroke (arrows).

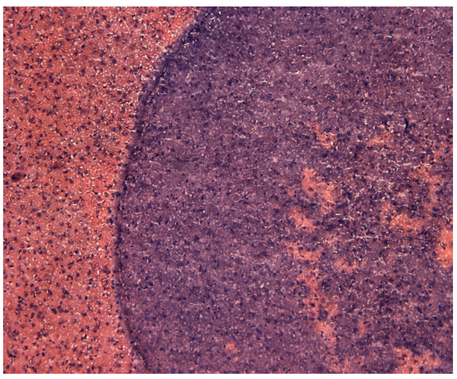

Figure 2. Chemical tissue corrosion. Note the dark blue color of the corroded tissue caused by a solution of 50/50 v/v ACN/0.1 TFA. There is a sharp curvilinear margin between the corroded tissue (right) and the undamaged tissue (left).

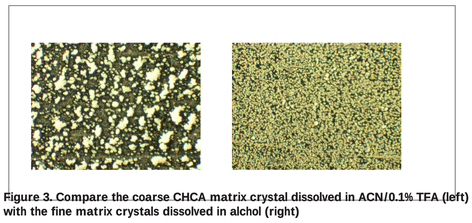

Figure 3. Compare the coarse CHCA matrix crystal dissolved in ACN/0.1% TFA (left) with the fine matrix crystals dissolved in alchol (right)

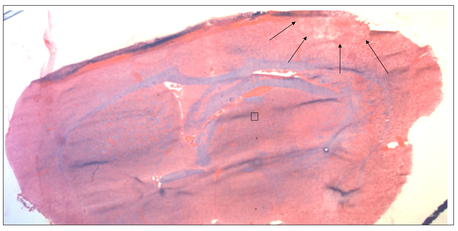

Figure 4. Histopathology 1 micron thick hematoxylin, eosin and Luxol fast blue stained coronal stroke murine brain section. Note the stroke in the upper right aspect of the tissue section, arrows. The tissue has been cryoprotected and there is no freezing artefact.

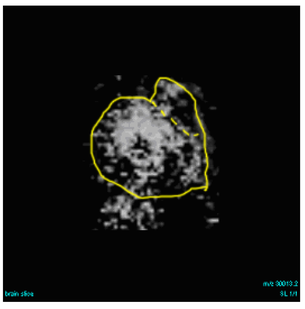

Figure 5. Imaging MALDI coronal brain image. Contiguous Imaging MALDI section from 1 micron thick coronal tissue section of murine brain (outlined). Note the region of irregularity in the upper right aspect of the image which corresponds to the stroke (outline with proximal dotted line) seen in in the histopathology, Figure 4. The resolution of this image while limited in comparison to the histopathology represents the future of more refined high resolution Imaging MALDI images.

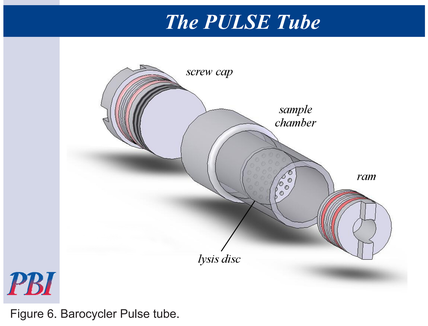

Figure 6. Barocycler Pulse tube.

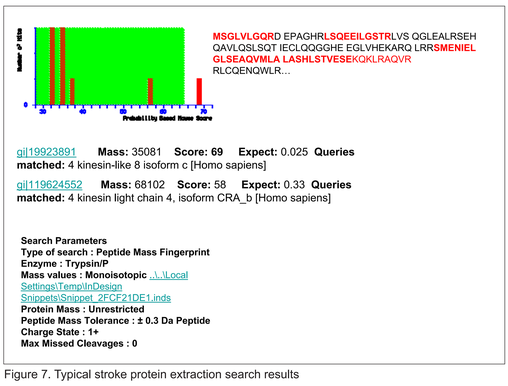

Figure 7. Typical stroke protein extraction search results

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