NYU Medical Center
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Stroke- Prophylactic Estrogen (Estradiol) Therapy

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Background and Significance

Cerebral ischemia immediately interdicts ATP production which affects MAP proteins, microtubule integrity, motor molecule processivity (specifically that of kinesin), and brings cellular metabolism to an abrupt stop. This year our laboratory was the first to report direct tissue MALDI identification of tubulin 24 hours post stroke in our murine stroke model.[1] Recently we demonstrated two kinesin isoforms, gi|19923891 4 kinesin-like 8 isoform, mw 35081, and gi|119624552 4 kinesin light chain 4 isoform CRA_b, mass 68102, 60 minutes after stroke induction with imaging MALDI (IMS). Estrogen is effective in reducing cerebral necrosis in stroke.[2] Estrogen was used to explore this protective effect. We also expanded our study of the metabolic cascade in stroke by high-pressure protein extraction from tissue, combined with a mineral oil organic solvent combination, and used HPLC for peptide separation.

Materials and Methods

Four groups of mice were studied. Group I was a control group with induced stroke and no treatment. Group II was given estrogen injections 12 hours pre-stroke, and immediately prior to stroke induction. Group III was given estrogen 12 hours pre-stroke. Group IV was given estrogen immediately before stroke induction A clinical neurological inventory developed by the authors was used to evaluate and compare the effects of estrogen therapy. The murine brains were harvested 60 minutes post-stroke induction.[3,4,5] Cryosections were obtained for IMS, hematoxylin and eosin histopathological staining, and protein extraction. Proteins were extracted from the brain with organic solvent and high pressure using ProteoSolve and the Barocycler respectively (Pressure BioSciences, West Bridgewater, MA). The protein fraction was trypsinized, and the peptides studied with LCMS (Hitachi NanoFrontier nLC, Dallas, TX).

Results

The peptide yield from the protein extraction tryptic digest identified new proteins not previously identified. The protein differences between the treated and untreated were small, however, there were significant differences between the treated and untreated animals. The estrogen treated animals had less neurologic deficit and less histopathologic change. (images not shown)

Table 1. Proteins from estrogen treated stroke tissue, A and B.
Proteins from untreated stroke tissue, C. There were both clinical and histologic differences between the treated and untreated animals.
There was less histopathologic change and less neurologic deficit in the estrogen treated animals.

A

Estradiol Treatment + Stroke
GI 32015, alpha-tubulin [Homo sapiens]
GI 200038, neurofilament-L
GI 193761, alpha-globin
GI 4507729, tubulin, beta 2 [Homo sapiens]
GI 1915913, Ulip2 protein [Mus musculus]

B

Estradiol Treatment + Stroke
GI 32015, alpha-tubulin [Homo sapiens]
GI 69885032, myelin basic protein isoform1 [Mus musculus]
GI 553919, alpha-1-globin
GI 6679937, similar to glyceraldeyde-3-phosphate dehydrogenase [Mus musculus]

C

Stroke
GI 32015, alpha-tubulin [Homo sapiens]
GI 69885032, myelin basic protein isoform 1[Mus musculus]
GI 45598372, brain abundant, membrane attached signal protein 1 [Mus musculus]
GI 84794631, tubulin, alpha-like 3 [Mus musculus]

Discussion and Conclusion

The combination of high pressure protein extraction with an organic solvent and LCMS increases the yield of peptides, and allows analysis of femtogram quantities of protein, significantly less than that required for 2D gel separation. Peptide separation by LCMS can provide identification of proteins from the extractions. These protein mass numbers can be used to reconstruct the images of the tissue with imaging MALDI, IMS, and localize the protein changes in the tissue. While there were only small protein differences between the treated and untreated animals, there were significant differences in neurologic deficit and histopathologic change in the tissue. Estrogen therapy protected the animals and decreased both the neurologic deficit, and the histopathologic stroke.

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