Comparison of Occlusion and Staining Techniques in Rodents with Induced CVA Injuries

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Background

Currently there are several stains that are used to visualize middle cerebral artery occlusions (MCAO) in rodents. These stains include 2, 3, 5 Triphenyltetrazolium Chloride, Fluoro-Jade B, NeuN, Cresyl-Violet, Nissl Stain, and Hematoxylin and Eosin. Methods for creating occlusions involve several suture techniques or use of a monofilament. Current staining techniques do not adequately visualize the area of damage post-MCAO. This led to the development of a new staining technique, ischemic contrast staining (ICS). ICS was developed to enhance visualization of MCAO.

Methods

Ten different peer-reviewed studies were analyzed to collect information about current standards of practice in research on middle cerebral artery occlusions. Databases used include EBSCOhost via SLU Library, Google Scholar, and PubMed. Articles were selected that met the inclusion criteria of examining MCAOs. Criteria that was considered, but not exclusionary, includes species used and age of animals. For comparative analysis, the method of occlusion, length of occlusion, staining technique, and time after occlusion staining occurred were studied. Thionine and HE were then compared to ICS to evaluate which is most effective for visualization of MCAO.

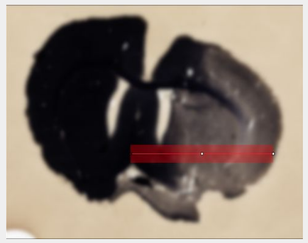

Figure 1a: ICS Sample

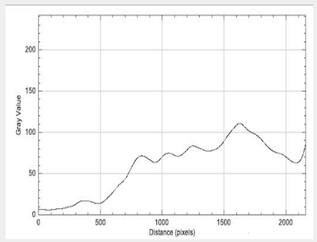

Figure 1b: ICS Plot

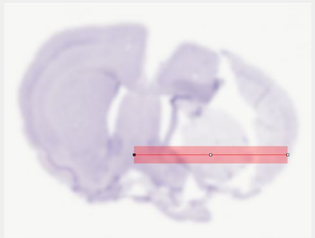

Figure 2a: Thionine Sample

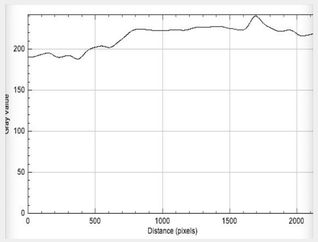

Figure 2b: Thionine Plot

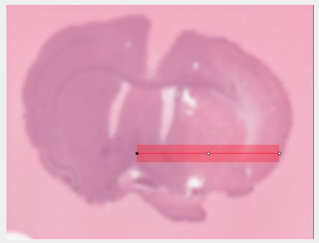

Figure 3a: HE Sample

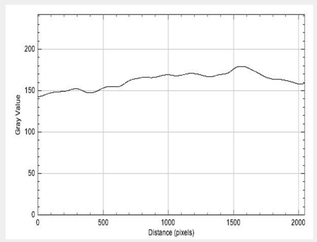

Figure 3b: HE Plot

Results

Chart 1: Comparison of Occlusion Method with Occlusion Time

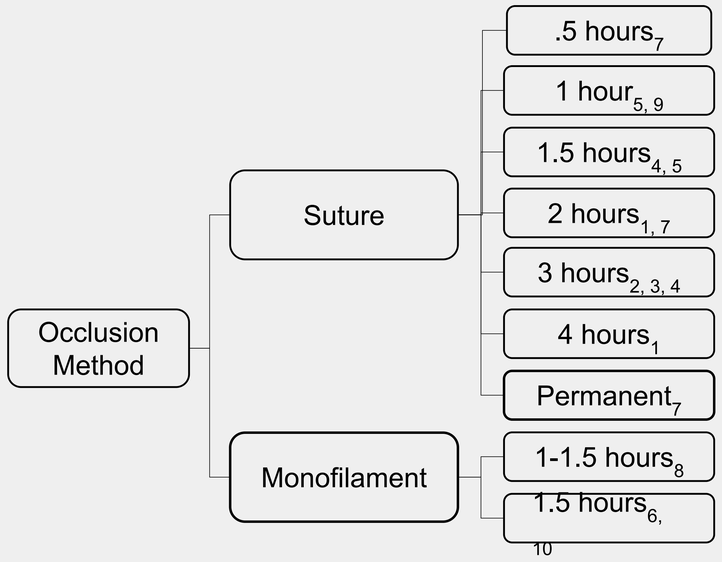

Chart 2: Comparison of Stain Type with Time Staining Occurred

Type of Stain	Studies Utilizing Stain	Time Elapsed Prior to Stain
2, 3, 5 TTC	1, 3, 6, 7, 8, 9 & 10	Immediately: 6 2 hours: 7 24 hours: 3,7,8,10 24-72 hours: 1 48 hours: 9 3 days: 10 7 days: 6,7,10 30 days: 7
Fluoro-Jade B	6	Immediately: 6 7 days: 6
Neu-N	6	Immediately: 6 7 days: 6
Cresyl-Violet	4 & 5	2-5 hours: 4 24 hours: 5
Nissl Stain	2 & 7	2 hours: 2, 7 24 hours: 2, 7 7 days: 7 30 days: 7
H & E	5	24 hours: 5

Discussion & Implications

A middle cerebral artery occlusion, MCAO, presents clinically as a cerebrovascular accident, CVA. According to the CDC, CVAs are the fifth leading cause of death for Americans. The high prevalence of CVA in society warrants research on how CVAs impact function. To complete this research, staining methods must be accurate and effective for comprehensive data collection. The new staining technique, ICS, differs from current standards of practice.

Conclusion

This comparative analysis of occlusion methods and staining techniques supports the continued development of the new method, ischemic contrast staining, to further evaluate the effects of MCAO in rodents.

References

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