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Preservative and Fixative Methods of Brain Biopsy- Review

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Abstract :
The objective of the study is to determine the preservative method and fixative methods for brain biopsies. A brain biopsy is a procedure used to remove a tumor or a piece of tissue from the brain so that it can be examined under a microscope to diagnose illness. As a variant of preservation, fixation has a dual role. Besides its main purpose to preserve cellular structures, it serves so called hardening of the specimen that is important for grossing manipulation. Hardening is an old term that stems from pre-formalin era of alcohol fixation. Actually fixation provides immobilization of the specimen's tissues by preventing them from moving during cutting procedure. In biopsies when differences between specimens by size and consistency are the most prominent, variations in fixation have practical significance. The modern biopsies processing includes many ancillary studies which require different modes of reservation. Fixation is only one though the most ubiquitous technique of preservation.

INTRODUCTION :

A brain biopsy is the removal of a small piece of brain tissue for the diagnosis of abnormalities of the brain , such as ALZHEIMER'S disease ,tumors, or inflammation/infections. By examining the tissue under microscope , the biopsy sample provided the information necessary to guide diagnosis and treatment. When an abnormality of the brain is suspected, Stereotactic (probing in three dimensions) brain needle biopsy is performed and guided precisely by a computer system to avoid serious complications. A small hole is drilled into the skull, and a needle is inserted into the brain tissue guided by computer-assisted imaging techniques (CT or MRI scans). Historically, the patient's head was held in a rigid frame to direct the probe into the brain; however, since the early 1990s, it has been possible to perform these biopsies without the frame. Since the frame was attached to the skull with screws, this advancement is less invasive and better tolerated by the patient. The doctor (pathologist) prepares the sample for analysis and studies it further under a microscope. The procedure is invasive and includes risks associated with anesthesia and surgery. Brain injury may occur due to removal of brain tissue. The resulting scar left on the brain has the potential to trigger seizures.

STORAGE METHODS OF BRAIN BIOPSY :

1.Pre- and Postmortem Conditions :

Autoradiographical analysis of postmortem human brain tissue or biopsies raises a series of methodological problems, some of which are also relevant for studies in animal models; others, however, are unique in experiments carried out with human brain sections and include both pre- and postmortem conditions.

The most important postmortem which has been in described in many reports , is the effect that neurological diseases can have on the distribution ,density, and affinity of specific neurotransmitter receptors. Therefore ,only the brains obtained from patients who died without a history of neurological or psychiatric disorders were used for chemoarchitectonic mapping of the human cerebral cortex. Furthermore, binding site density and affinity can also be affected by aging depending on the receptor type under consideration. A consistent finding is the age-related decrease in the density of glutamate NMDA receptors, which seems to be accompanied by regionally specific changes in the interaction between glutamate and other neurotransmitters such as dopamine and GABA.

The most common technique in preservation is deep freezing method.

DISADVANTAGE: The effect of postmortem delay in the freezing of brain tissue as well as of prolonged storage of the frozen tissue prior to analysis on receptor binding assays are potential artifacts that may limit interpretation of the effects of disease on receptor populations. However, only a relatively small number of reports discuss the problems caused by binding sites increased with increasing postmortem delay of freezing. Prolonged storage of deep frozen tissue is inevitable when analyzing a statistically significant sample of human brains.

2.Tissue Processing :

Both the quality of receptor autoradiographs and the preservation of histological stainings are highly dependent on the handling of the brain immediately after biopsy. Although fixation of the brain before deep freezing and cutting clearly improves the quality of histological stainings,it also impairs the structure of receptor proteins and leads to changes in specific and non-specific binding, altering the ratio between both parameters to different degrees. Therefore, we use only unfixed ,deep frozen brains for receptor autoradiography.

Immediately after biopsy, the brain were photographed and the hemisphere and brain stem were seperated and stored in plastic bags on crushed ice before further dissection. Each hemisphere was cut into coronal, saggital ,or horizontal slabs, which were placed on a sheet of strong aluminium foil to preserve a flat sectioning surface and to avoid distortion. This method was enabled fast freezing of the brain tissue ,avoiding freeze artifacts such as the appearance of ice crystals, which would destroy cellular morphology . The tissue was then stored in a deep freezer at -70 degree in air tight bags to protect it from freeze-artifacts.

3.Labelling Procedure:

The brain tissue was serially sectioned in a cryostat microtome for large sections into 20-um sections at -20 degree. The sections were thaw-mounted on gelatin –coated glass slides and freeze-dried overnight. Alternating sections were incubated with tritiated ligands alone or with the tritiated ligands and a receptor type-specific displacing agent. Non-specific binding is taken into consideration only when it amounts to more than 10% of the total binding sites marked by the ligand. Therefore, the exposure time is adjusted for each ligand accordingly to this constraint. During image acquisition ,a shading correction is carried out.

FIXATION METHODS OF BRAIN BIOPSY :

Fixation Methods

The brain will begin to deteriorate as soon as its blood supply is interupted. The deterioration is rapid, and it is, therefore, important to arrest the deterioration as quickly as possible. The process of preserving the brain is called "fixation".

The two most common ways of fixing the brain are freezing and the use of fixative solutions. Each method has its advantages.

Freezing

Freezing is a much quicker way of fixing the brain and tends to preserve more of the brains biochemistry, but the tissue is trickier to deal with as it must remain frozen until it is on the microscope slide. There is much blood left in the tissue. Freezing is not appropriate if you intend to do immunocytochemistry. It is good for glycogen phosphorylase staining, AChe staining, Mao staining, and Nissl staining (cresyl violet or thionin). Frozen sections can be mounted on plain glass microscope slides.

In the study on brain fixation done by STEVE MILWAY et al, the brain of the rat was frozen by placing it in the freezer or in the chamber of the cryostat-microtome, but for best results rapid freezing was done. Two common methods of rapidly freezing the brain are dry ice and liquid nitrogen.

In the lab they used methyl butane (isopentane) cooled to -72 degrees in a ultra cold freezer. A covered jar of methyl butane was placed in the freezer 20-30 mins prior to sacrificing the rat. The rat was then anaesthetized and decapitated. The brain was quickly removed from the skull and immediately immersed in the cold methyl butane. The brain was frozen within a few seconds. It was then wrapped in aluminium foil and stored in the freezer until sectioning.

Fixative Solutions

The most commonly used fixatives were formalin, glutaraldehyde, and alcohol . A 10% formalin solution are adequate for most purposes, but many stains require specialized perfusions where pH and osmolarity are carefully controlled.

Fixing the brain with fixative solutions takes longer, but the brain is much more resilient and easier to handle. When perfused, most of the blood are removed from the tissue. Fixative Solution are used for immunocytochemistry, HRP staining, and biocytin staining. Nissl stains were also used on tissue fixed in fixative solutions. Fixative solutions are also used if the tissue is going to embedded in paraffin or plastic and for vibratome sectioning. Tissue fixed with fixative solutions should be mounted on gelatin coated microscope slides if the sections will be processed after the tissue is on the slides.

Tissue can be fixed in two ways: by immersion and by perfusion. Immersion is suitable only for small or thin pieces of tissue. In the immersion method the tissue is simply soaked in the fixative solution. The immersion method is often used to fix frozen section that have been mounted on slide. The slides are dipped in formalin or alcohol for a few minutes to fix them after sectioning and staining. Immersion is not a suitable fixation for larger pieces of tissue such as a whole brain. The outside of the brain fixes and impedes the penetration of the fixative to the centre of the brain. For whole brain, the perfusion method is used. In the perfusion method, the circulatory system is cleared of blood and used to circulate the perfusants uniformly through the brain and body.

CONCLUSION :

In this review we discussed various preservative and fixative methods of brain biopsy. The preservative or storage methods are pre-and postmortem conditions, tissue processing and labeling procedure. The fixative methods are freezing, immersion and perfusion. The best preservative and fixative method are tissue processing and freezing method. As freezing the brain tissue hasten the fixation and provides immobilization of the specimen by preventing them from moving during cutting procedure, it became a best method of preservation and fixation. Demonstration by Steve Milway on rat brain describes the freezing method. This can be followed in routine human brain biopsies also. Fixation is only one though, the most ubiquitous technique of preservation, that helps in better understanding of the brain lesions and provides better results in ancillary studies which are the most targeting research areas in Neuropathology.

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