MICROWAVE STIMULATED FIXATION AND RAPID PROCESSING OF TISSUE FOR HISTOPATHOLOGY

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ABSTRACT

A rapid method for obtaining high quality paraffin sections is described. This method enables preparation of paraffin blocks in about 20 minutes and is based on microwave stimulated tissue fixation and processing. A total of 200 tissues were processed. Tissue blocks of size 1x1x0.5 were found ideal for processing. Three protocols were evaluated for tissue processing using different timings of exposure to graded alcohols, chloroform and wax. It was observed that utilizing an additional step of exposure to 70 per cent alcohol during tissue dehydration resulted in preparation of paraffin blocks which were light microscopically excellent and highly comparable to conventional methods.

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KEY WORDS : Microwaves; Tissue fixation; Histopathology.

Introduction

Tissue diagnosis is of paramount importance in instituting prompt and definitive treatment especially in cases of malignancy where immediate management can now offer longer lease of life and in some cases even cure. A need for rapid method for tissue fixation and processing in histopathology was therefore felt and with the emergence of the microwave (MW) diagnosis in surgical pathology was revolutionized. The concept of fixation of tissues using microwaves was first advocated by Meyers in 1970 [1]. The MWs can penetrate tissues to a greater extent than heat waves. They cause bipolar molecules like water to rapidly oscillate producing instantaneous heat. This molecular agitation coupled with other interactions at the cell membrane result in tissue fixation and can also be utilized for diffusion of chemicals into tissues for rapid processing for histopathology [2,3]. The time honoured conventional procedure in our service laboratories today takes about five days for a final tissue diagnosis, whereas with MW stimulated technique, it is possible on the same day.

Material and Methods

Tissues (both biopsy and necropsy) were processed by the microwave stimulated method. Specimens were mostly received in 10 per cent formol saline as precaution against autolysis due to delay in transit. However some tissues were also collected in isotonic saline. Tissues from necropsy were processed and evaluated taking into consideration the time of death, the conduct of necropsy and the state of preservation of body. Tissue blocks were prepared in 3 sizes i.e. 1x1x1 cm (n=40), 1x1x0.5 cm (n=120) and 0.5x0.5x0.5 cm (n=40).

The glass jar containing tissues was placed in a commercial microwave oven (make Essentia Classic) and irradiated for 120 seconds at full power (700 watts) so as to reach a temperature of 58 degrees centigrade. This fixed the tissue. Such tissues fixed in isotonic saline and the other tissues received in 10 per cent formol saline after conventional fixation were subjected to MW stimulated rapid processing by one of the three protocols as shown in Table 1. The protocols of tissue processing were based on observations published by Bøon

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et al [2]. Throughout the tissue processing protocol the MW oven was set at 50 per cent power.

TABLE I
Protocol for MW stimulated processing

Reagent used	Protocol 1	Protocol 2	Protocol 3
70% alcohol	—	—	4 mins
100% alcohol	5 mins	10 mins	5 mins
Chloroform	5 mins	5 mins	6 mins
Wax	5 min	5 min	5 min
Total tissue	50	50	100

The tissue was subsequently embedded in molten wax. 3-5 micrometre thick tissue sections were obtained and stained by haematoxylin and eosin (H&E). Identical sets of tissue slices were taken as controls simultaneously with the MW induced tissue processing. These tissue slices were processed by conventional methods. Tissue sections were similarly obtained and stained using H&E stain. The quality of the section and the staining obtained from the tissue blocks obtained by the various protocols was then compared, keeping the following points into consideration :

(a) Preservation of tissue architecture

(b) Nuclear and cytoplasmic staining characteristics

Results

A total of 200 tissues were processed by the MW stimulated method. This included 100 biopsy

Fig. 1 : Protocol 3 : Ideal tissue processing by MW stimulated method. The results are indistinguishable from conventional tissue processing.

and 100 necropsy tissues. Analysis of H&E stained sections revealed that sections of tissue blocks processed using the MW stimulated method by protocol 3 were virtually indistinguishable from conventionally processed tissues (Fig 1). Furthermore, the time needed to prepare the paraffin block by this method was only about 20 minutes. The tissue architecture was well preserved and no significant difference between nuclear size, shapes and staining characteristics were discernible. The cell cytoplasm in 47 cases showed a deeper eosinophilia. Twenty five tissues had been received in isotonic saline and microwave processed within 4 hours of removal from the body. None of the tissues showed autolytic change as demonstrated in Fig 2. Tissue sections in 16 of these cases showed lysis of erythrocytes in the tissues. This was not seen in those cases where the tissue had been exposed to 10 per cent formal saline for at least an hour prior to irradiation. This was roughly the time gap between the surgical procedure and the tissue sampling from the gross specimen in the laboratory. This short exposure to formalin, however, is not adequate to fix tissues.

The ideal block size for tissue processing was 1x1x0.5 cm (n=120). Thicker slices of the size 1x1x1 did not process well. Tissues of size 0.5 x 0.5 x 0.5 cm (n=40) processed well, however, this size was not favoured since the tissue sampled was small. Table I shows the tissue processing protocol. The 'ideal' MW stimulated tissue processing was obtained by protocol 3 (Fig 1). The results obtained by protocol 1 were unsatisfactory as dehydration of the central region of the tissue was

Fig. 2 : Autolytic changes due to improper fixation.

Fig. 3: Protocol 1 : improper dehydration in the central region of the tissue.

inadequate (Fig 3). Using protocol 2, the section were observed to be very dense and tissue clearing was improper (Fig 4). The histopathological sections obtained by protocol 3 were virtually indistinguishable from those obtained by the conventional methods.

Discussion

The microwaves are electromagnetic waves of a frequency of 2.45 GHz. They can rapidly penetrate biological tissues. Microwave induced excitation of molecules in tissues is a process in which the applied energy penetrates at a much greater depth compared to infrared rays. The radiation causes bipolar molecules such as water to oscillate through 180 degrees at a rate of 2,450,000,000 times per second. This agitation generates instantaneous heat which is proportional to the energy flux and is continuous until the radiation ceases. It has been implied that the heat generated by molecular movement may be responsible for the process of fixation. In addition, other modes of physical interaction at the cell membrane may occur as a result of oscillating electric and magnetic fields [2,3]. Alterations in macromolecular hydrogen bonding, proton tunnelling and disruption of bound water may induce changes in biologic systems [3]. Hopwood et al [4] suggested that fixation by microwave appear to depend upon denaturation of proteins with subsequent formation of disulphide bonds between the unravelled molecules resulting in decrease in solubility.

As the thickness of the tissue block sampled is

Fig. 4: Protocol 2 : Dense central region and inadequate clearing.

0.5 cm, the process of MW excitation is uniform through out the tissue. This is in contrast to simple heating where the energy distribution is not uniform. This process can be completed in 120 seconds of irradiation in isotonic saline and is free of noxious fumes [5]. The conventional method of tissue processing is time consuming. The procedure will take anything from an overnight cycle to 24 hours. It is obvious that the hours spent for fixation and processing result in a palpable time gap between the surgical biopsy and the tissue diagnosis. The use of frozen sections in histopathology was a major landmark achieved by the pathologists to help the surgeons to reach a quick diagnosis, but with the passage of time, the procedure exposed its limitations.

It has been noted in medical literature that tissues fixed by MW irradiation show lysis of erythrocytes. This phenomenon is not observed in tissues that had been immersed in buffered formalin for a brief period prior to irradiation [3,6]. A similar observation has been made in our study also. A brief exposure to formalin is useful, and roughly corresponds to the time lapsed between tissue sampling and the surgical procedure. Therefore, tissues should always be despatched in formol saline to protect against autolysis due to accidental delay in transit and also to prevent lysis of erythrocytes during processing. This short exposure to formalin, however, is inadequate for complete tissue fixation.

MW irradiation has also been utilized to enhance diffusion of chemicals into tissues. This forms the basis of the MW stimulated rapid

method of processing tissues for histopathology. The entire process can be completed in 20-30 minutes, which otherwise takes hours by conventional methods. The histopathological sections obtained by the two methods show no significant differences.

In all 200 tissue blocks were prepared and examined. Good results were obtained which were comparable to those obtained by conventional methods. The time needed to prepare a paraffin block by the MW stimulated method was approximately 20 minutes. This makes the MW stimulated tissue processing especially valuable in cases where a rapid diagnosis is required and a frozen section is inconclusive. The use of formalin with its noxious fumes and potentially toxic and carcinogenic effects can be avoided [7-9]. In addition, this innovative method proves its worth since the only apparatus required to prepare tissue blocks is a commercially available MW oven. It is to be ensured that only plastic-ware or plain glassware is used in MW stimulated tissue processing as microwaves do not penetrate metals. The tissues should always be completely immersed in respective reagents during the process. A precise and optimum energy and time control should be used to avoid tissue damage and boiling of reagents. This tech-

nique is certain to help the clinicians in instituting prompt and appropriate treatment as an early final diagnosis is available.

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