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Staining reactions of microwave processed tissues compared with conventional paraffin wax processed tissues

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ABSTRACT

The staining quality of microwave processed tissues was compared with the staining quality of the conventional paraffin wax processed tissues. Normal lung, kidney, liver, intestine and heart tissues obtained from an adult Wistar albino rat were fixed in 10% formol saline and processed by the conventional paraffin wax method. The twin specimens were also processed by the microwave method. Sections were cut with the rotary microtome, paired and stained by heamatoxylin and eosin method for general tissue structure, Weigert's van Gieson iron haematoxylin for collagen fibres, Verhoeff's van Gieson iron haematoxylin for elastic fibres, periodic acid Schiff reaction for neutral mucopolysaccharides, Gordon and Sweet's method for reticular fibres, Alcian blue (pH2.5) for carboxylated and sulphated mucopolysaccharides, Congo red method for amyloid, Masson's trichrome for collagen and muscle fibres and Gomori's aldehyde fuchsin method for elastic fibres. Microscopically, there were no significant differences in the staining reactions of all the techniques when they were compared with the microwave processed tissues. Nuclear, cytoplasmic, extracellular and intracellular materials appeared the same with the conventionally processed tissues by the paraffin wax method. Muscles, collagen fibres, elastic fibres and reticular fibres were also stained the same way as the conventionally processed tissues. Neutral carbohydrates and amyloid were equally of the same appearance with the conventional paraffin wax method. However, there was significant tissue shrinkage in the conventional method which was not apparent microscopically. Microwave method is faster, cheaper and prevents the use of xylene which is harmful to humans

Key words: microwave, paraffin wax, staining, tissue processing.

INTRODUCTION

Tissue processing is the act of preparing tissues for microtomy. Several methods exist. They include paraffin wax, low viscosity nitrocellulose, celloidin and several forms of resins [1]. In the paraffin wax tissue processing method, tissues can be processed rapidly, manually, with an automatic processor [2] or with the use of a microwave [3,4]. The method of choice depends on the urgency of the specimen, availability of materials and availability of equipment. The overall aim of tissue processing is to allow for production with the use of the microtome, very thin sections of tissue which can be examined under the microscope because only thin sections allow light to pass through them which is necessary for microscopy [1]. Sample preparation for microscopy is based on physical and chemical processes. These processes can be influenced by microwave irradiation [5]. Microwaves are non-ionizing radiations with electromagnetic properties. Their frequencies range from 300 MHz to 300 GHz and wavelengths from 1 mm to 1 m. All domestic microwaves operate at 2.45 GHz, corresponding to a wavelength in vacuum of 12.2 cm [3]. Microwave excitation of molecules is a process in which applied energy penetrates into the tissues to a greater depth as compared to the other manual methods of tissue processing [6]. Dipolar molecules, which are present in the field, are forced to oscillate and this leads to an increase in thermal agitation [3]. The kinetic energy thus generated is converted into heat energy which is utilized in the microwave technique. The aim of this work was to compare staining reactions of sections processed by the conventional method with sections processed by the microwave technique.

MATERIALS AND METHODS

An adult Wistar albino rat was sacrificed through cervical dislocation and carefully dissected. Sections were taken from the lungs, kidney, liver, intestine and the heart. The sections were fixed in 10% formol saline for 24 hours. Each section was further cut into two sub sections of about 3mm x 3mm x 3mm. The first group consisting of sub sections of lungs, kidney, liver, intestine and the heart was processed by the conventional routine paraffin wax method by dehydrating in ascending grades of alcohol of 70% alcohol, 95% alcohol and 2 changes of absolute alcohol for 2 hours each. Sections were then cleared in 2 changes of xylene, infiltrated with 2 changes of paraffin wax at 70ºC for 2 hours each. Sections were finally embedded in paraffin wax and cut at 4µm with a Rotary microtome. They were floated on water, picked with slides and dried at 50ºC for 30 minutes. The second group of sub sections was processed by the microwave technique: tissue sections were rinsed in running water for 5 minutes, dehydrated in 100% ethanol for 15 minutes at 65ºC in a microwave. Sections were cleared in 100% isopropanol for 10 minutes at 74ºC in the microwave, infiltrated in 3 changes of liquid paraffin wax for 5 minutes at 65ºC in the microwave with boiling chips, 5 minutes at 74ºC in microwave with boiling chips. Tissues were transferred into another change of liquid paraffin for 5 minutes at 82º in microwave with boiling chips. Tissues were finally transferred onto cassettes containing clean paraffin wax and embeded. Tissues were cut at 4µm with a Rotary microtome, floated on water, picked with slides and dried at 50ºC for 30 minutes. Paired sections from the conventional paraffin wax method and the microwave technique were stained by the following methods: heamatoxylin and eosin method for general tissue structure, Weigert's van Gieson iron haematoxylin for collagen fibres, Verhoeff's van Gieson iron haematoxylin for elastic fibres, periodic acid Schiff’s reaction for

neutral mucopolysaccharides, Gordon and Sweet's method for reticular fibres, Alcian blue (pH 2.5) for carboxylated and sulphated mucopolysaccharides, Congo red method for amyloid, Masson's trichrome for collagen and muscle fibres and Gomori's aldehyde fuchsin method for elastic fibres.

Heamatoxylin and Eosin Method for General Tissue Structure

Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were stained in Erhlich's haematoxylin for 15 minutes, rinsed in water, differentiated in 1% HCl in 70% alcohol for 1 minute, rinsed in water, blued in Scott's tap water substitute for 2 minutes, counterstained with 1% eosin for 1 minute, rinsed in water, dehydrated, cleared and mounted in DPX.

Weigert's van Gieson Iron Haematoxylin

Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were stained in equal volumes of Weigert's haematoxylin solutions A and B for 20 minutes, rinsed in water, differentiated in 1% HCl in 70% alcohol for 1 minute, rinsed in water, blued in Scott's tap water substitute for 2 minutes, then with 95% alcohol for 5 minutes. Sections were counterstained with van Gieson for 2 minutes, dehydrated, cleared and mounted in DPX.

Verhoeff's van Gieson Iron Haematoxylin

Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were stained in freshly prepared Verhoeff's Iron Haematoxylin solution for 20 minutes, rinsed in water, differentiated in 2% ferric chloride until elastic fibres remained black, washed in tap water for 10 minutes, then with 95% alcohol for 5 minutes. Sections were counterstained with van Gieson for 2 minutes, dehydrated, cleared and mounted in DPX.

Periodic Acid Schiff Reaction for neutral mucopolysaccharides

Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were oxidized in 1% periodic acid for 5 minutes, washed in water, then in distilled water. Sections were then stained in Schiff's reagent for 10 minutes, washed in tap water for 15 minutes, counterstained with Mayer's haematoxylin for 5 minutes, rinsed in water, differentiated in 1% acid alcohol for 5 seconds, blued in Scott’s water substitute for 10 minutes, dehydrated, cleared and mounted in DPX.

Gordon and Sweet's Method for Reticular Fibres

Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were oxidized in acidified potassium permanganate for 5 minutes, washed in distilled water, then in tap water. Sections were then mordanted in 2.5% iron alum for minutes, washed in 3 changes of distilled water, transferred to silver solution until sections became transparent, washed in distilled water, reduced in 10% formalin for 1 minute, washed in tap water, then with distilled water, toned in 0.2% gold chloride until section turned a purplish colour, washed briefly with distilled water, then fixed in 5% sodium thiosulphate for 5 minutes, washed in water for 1 minute and counterstained in 1% neutral red for 1 minute. Sections were finally dehydrated, cleared in xylene and mounted in DPX.

Alcian Blue (pH 2.5) for Carboxylated and Sulphated Mucopolysaccharides
Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were stained in Alcian blue pH 2.5 solution for 30 minutes, rinsed in distilled water and then washed in tap water for 5 minutes, counterstained with 1% neutral red for 30 seconds and rinsed in water. Sections were finally dehydrated, cleared in xylene and mounted in DPX.

Congo Red Method for Amyloid
Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were stained in Congo red staining solution for 5 minutes, differentiated in alcoholic potassium hydroxide until excess Congo red was removed. Sections were stained in Mayer's haematoxylin for 2 minutes and blued in tap water. Sections were finally dehydrated, cleared in xylene and mounted in DPX

Masson's Trichrome for Collagen and Muscle Fibres
Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were stained in equal volumes of Weigert's solutions A and B for 30 minutes, rinsed in water, differentiated in 1% acid-alcohol until only nuclei were stained, wash in water for 10 minutes and stained in acidified ponceau 2R for 5 minutes, rinsed in distilled water, differentiated and mordanted in 1% phosphomolybdic acid for 5 minutes, drained slide and stained in acidified light green for 5 minutes. Sections were differentiated in 1% acetic acid for 1 minute, blotted, dehydrated in absolute alcohol, cleared in xylene and mounted in DPX.

Gomori's Aldehyde Fuchsin Method for Elastic Fibres
Sections were dewaxed in xylene and hydrated through absolute alcohol and 70% alcohol. They were put in Lugol's iodine for 20 minutes, rinsed in water and transferred to 5% sodium thiosulphate for 3 minutes, washed in water, rinsed in 70% alcohol and stained in acidified aldehyde - fuchsin solution for 5 minutes, rinsed in 70% alcohol and counterstained with light green. Sections were finally dehydrated, cleared in xylene and mounted in DPX.

RESULTS

Macroscopy
There was an observable shrinkage in the tissues processed by the conventional paraffin wax method when compared with the microwave processed tissues. The omission of xylene in the process makes it less harmful to humans. It also makes it cost effective. Microwave is also faster than the conventional paraffin wax method.

Microscopy
Quality of staining was the same in both the microwave and the conventional methods. Staining reactions of the nuclei, cytoplasm, intracellular, extracellular substances, muscle fibres, collagen fibres, elastic fibres, neutral and acidic carbohydrates, amyloid and general tissue structure were the same in both methods. Nuclear and cytoplasmic staining in haematoxylin and eosin technique were slightly brighter than in the conventional methods but could not be regarded as significant.

DISCUSSION

Microwaves were first introduced as a method of fixation to accelerate the action of cross-linking fixatives and latter to greatly accelerate the various stages of tissue processing to produce a paraffin block in 30 minutes [7]. An extensive range of ultrafast microwave stimulated special stains has been developed, and immunohistochemical procedures could be completed in 20 minutes by employing microwaves [7]. He stated further that cellular antigens were distinctly better preserved in tissues fixed by microwaves than by conventional cross-linking fixatives [7]. The basic effect of microwave irradiation is stimulation of diffusion and enhancement of reaction rates with internal heating being the key element in the process [5]. Heating by conventional means can also be used for tissue processing, but the results obtained are markedly inferior to that in microwave processing. The suggested reason is that in conventional method of heating, the heat might not be uniformly distributed throughout the tissue [8]. The aim of cytochemical techniques is to localize specific biochemical components in particular tissue and cell compartments. However, since preparation of tissues for structural observation results in major alterations of the properties of their components, a major problem is to retain an adequate degree of their biochemical properties as well as adequate structural preservation [9]. The histologic quality of the microwave histoprocessing was compared with that of conventional method and to determine its positive impact on turnaround times and reduction of costs of tissue processing [10]. They concluded that overall, the quality of microscopic tissue from both methods was identical. Microwave processing shortened the time of processing without compromising the overall quality of the histologic section and was cost-effective [10]. The quality of microscopic image of cryostat sections that had been subjected to microwave assisted fixation was compared with that resulting from conventional air drying of the sections [11]. The role of microwaves in producing rapid special stains on cryostat sections was also assessed. They found that methods such as periodic acid Schiff, alcian blue, Gordon and Sweets's reticulin, Masson Fontana, Elastica, Prussian blue and Van Gieson could be performed within three minutes of cutting a cryostat section and that the cytological detail of nuclei was much clearer using the microwave technique, allowing more accurate determination of cell type. The microwave oven seems to have major potential in improving the diagnostic accuracy of surgical frozen sections [11]. In this experiment, the effect of staining on microwave processed tissues was determined. No significant effect was observed microscopically between tissues processed by the paraffin wax method and tissues processed with the microwave. However, there was significant tissue shrinkage in the conventional method which was not apparent microscopically. Microwave method is faster, cheaper and prevents the use of xylene which is harmful to humans

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