Comparison of Tissue Preservation using Formalin and Ethanol as Preservative Formula

See Woan Shian,[1] Arifin Soenggono,[2] Sawkar Vijay Pramod[3]
[1]Faculty of Medicine Universitas Padjadjaran, [2]Department of Anatomy and Cell Biology, [3]Department of Surgery Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung

Abstract

Background: Tissue preservation can be performed through embalming, by providing the chemical embalming fluid to the human remains. Formalin's preservative formula is the foundation for modern methods of embalming. Unfortunately, this preservative formula has several disadvantages. While Ethanol's preservative formula is a considerable agent to replace formalin's preservative formula. The aim of this study was to compare the tissue preservation using formalin and ethanol as preservative formula.

Methods: This study was carried out from September–October 2014 in the Laboratory of the Department of Anatomy, Faculty of Medicine, Universitas Padjadjaran. The study used the laboratory experimental method with consecutive sampling of 16 Wistar Rats. Thirty two soleus muscles and thirty two colons were collected and divided into two groups. Each group consisted of 16 soleus muscles and 16 colons. Group 1 was preserved with formalin's preservative formula and Group 2 was preserved with ethanol's preservative formula. The two groups were preserved for six weeks. The tissue's color, consistency, odor and the growth of bacteria were determined before and after treatment.

Results: Tissues preserved with ethanol's preservative formula had better tissue preservation in the aspect of color and odor, compared with formalin's preservative formula. Both preservative formulas showed no growth of bacteria in tissues but failed to retain the consistency. All the data were analyzed with Chi-square test.

Conclusions: Ethanol's preservative formula preserves better quality of tissue compared to formalin's preservative formula. **[AMJ.2016;3(3):359–63]**

Keywords: Ethanol, formalin, tissue preservation

Introduction

In the framework of undergraduate medical education, cadavers are main educational tools which are intended for dissection and to demonstrate prosected specimen through visual, auditory and tactile pathways.[1,2] Hence, tissue preservation plays a pivotal role which is to preserve cadavers, maintaining its life-like physical characteristics and prevents its decomposition.[3] This can be done through embalming, which is an art and science in modern culture by giving the embalming fluid which is composed of chemical to the human remains.[3] The aims of embalming for anatomical purposes are to prevent putrefaction progress on the cadavers, ensure that there is no risk of infection on contact with dead body, prevent over-hardening and retention of color of tissues and organs, prevent desiccation, inhibit fungal or bacterial growth and has lesser risk of being a potential environmental chemical hazards and biohazards.[3,4]

Formalin, which is composed of a saturated water solution containing 39–40% of formaldehyde, is discovered in the year 1869.[3] After formalin was determined to be an excellent preservative, it became the foundation for modern methods of embalming.[3,4] However, formalin as preservative formula has several disadvantages for embalming purposes. Formalin's preservative formula will lead to health problems, causes over hardening of tissues, coagulates blood, convert tissues to a grey hue when it mixes with blood, fixes

Correspondence: See Woan Shian, Faculty of Medicine, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km.21, Jatinangor, Sumedang, Indonesia, Phone: +6287822004420 Email: seewoanshian@gmail.com

discolorations, dehydrates tissues, constricts capillaries and has a suffocating odor.[2,3,5,6] In addition, ethanol has been phased out for Product Type 22 'Embalming and taxidermist fluids' by 1 September 2006.[3] Based on several researches, ethanol has several advantages as preservative formula and has less risk to health problems.[7] Therefore, ethanol can be considered to replace formalin as preservative formula.[3] This study was conducted to compare the tissue preservation using formalin and ethanol as preservative formula.

Methods

This study was carried out from September–October 2014 in the Laboratory of Department of Anatomy, Faculty of Medicine, Universitas Padjadjaran. All experiments performed on the laboratory animals in this study were approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran. Formalin and ethanol preservative formula were obtained from the Laboratory of Department of Anatomy, Faculty of Medicine, Universitas Padjadjaran.

The study used the laboratory experimental method with consecutive sampling of sixteen healthy male Wistar Rats as study subjects. The inclusion criteria for the study subjects were healthy Wistar rats which were 8 weeks old male and weighing between 250g, whereby the exclusion criteria was the Wistar rats which did not move actively.

The preservative chemicals were prepared one week before the dissection by measuring the preservative chemicals according to the volume using a measurement beaker and beam balance. The formalin's preservative formula consisted of 150ml of formalin, 200ml of glycerin, 50ml of phenol, 200g of sodium chloride and 600ml of water whereas the ethanol's preservative formula consisted of 700ml of ethanol, 200ml of phenol, 40ml of glycerin, 10g of sodium chloride, 30ml of water and 30ml of formalin.

Then, the Wistar rats were dissected to collect 32 soleus muscles and 32 colons. Firstly, a Wistar rat was put into an inverted beaker for anesthesia. The inverted beaker consisted of cotton that was soaked with the lethal volume of ether. Next, the Wistar rat was placed on a dissecting tray with needles to secure it. Then, the dissection started by cutting down from the neck to the lower abdomen. Another two lines were cut towards left and right from the end of the center line. The visceral organs were removed and the blood was washed with NaCl 0.9%. Afterward, the soleus muscles and colons were collected. The dissection procedure was repeated for all the Wistar rats.

After all the tissue samples were collected, the soleus muscles and colons were divided into two groups. Group 1 was preserved with formalin's preservative formula and Group 2 was preserved with ethanol's preservative formula. Each tissue was preserved with 6ml of preservative fluid in one plastic container. The two groups were preserved for six weeks in a temperature of 10^0C. The tissue's color, consistency, odor and the growth of bacteria were determined before and after the preservation.

The colors of the tissues were accessed visually, the odors of the tissues were accessed by smelling and the consistencies of the tissues were accessed by tactile sensation. The growths of bacteria of the tissues were determined by the results on blood agar. The

Table 1 Color of Tissue Before and After Preservation

Color	Formalin's Preservative Formula	Ethanol's Preservative Formula	p-value (Chi-square Test)
Before			0.599
Pink	16	16	
Pale Red	16	16	
After			0.000
Grayish Chocolate	16	0	
Reddish Pink	0	16	
Grayish White	16	0	
Yellowish White	0	16	

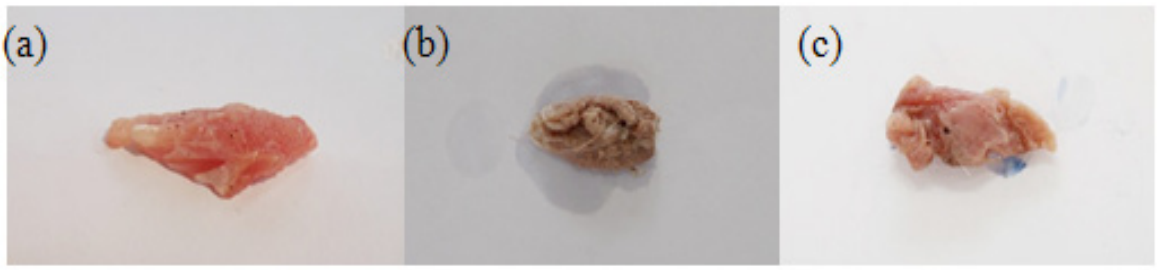

Figure 1 Color of Soleus Muscle: (a) soleus muscle before preservation showed pink color. (b)soleus muscle preserved by formalin's preservative formula preservation showed grayish chocolate color. (c)soleus muscle preserved by ethanol's preservative formula preservation showed reddish pink color.

Figure 2 Color of Colon: (a) colon before preservation showed pale red color. (b)colon preserved by formalin's preservative formula preservation showed grayish white color.(c)soleus muscle preserved by ethanol's preservative formula preservation showed yellowish white color

procedure of detection of the growth of bacteria began by putting the tissue samples into test tubes which contained brain-heart infusion media. After the samples were incubated at a temperature of 37^0C for 24 hours, each sample was inoculated on blood agar. Then, results were obtained after the incubation of blood agar for 24 hours at a temperature of 37^0C.

Furthermore, data of color, consistency, odor and growth of bacteria of the tissues before and after preservation were statistically analyzed using the Chi-square test. Statistically significant was considered when $p<0.05$. Analysis was performed by comparing the tissue preservation between the formalin's preservative formula group and the ethanol's preservative formula group.

Results

The comparison of color of the tissues before using formalin and ethanol as a preservative formula had no significant difference because the p-value was more than 0.05.

However, for the comparison of color of the tissues after using formalin and ethanol as a preservative formula, it had a significant difference because the p value was less than 0.05. The color of the tissue that was preserved by the ethanol's preservative formula was more similar to the color of the tissue before preservation rather than the formalin's preservative formula. Thus, the color of the tissue preserved by the ethanol's preservative formula was better than the color of the tissue preserved by the formalin's preservative formula (Table 1).

The comparison of odor of tissue preservation after using formalin and ethanol as a preservative formula had a significant difference because the p-value was less than 0.05. The odor of tissue preserved by ethanol's preservative formula was better than the odor of tissue preserved by formalin's preservative formula (Table 2).

The comparison of consistency of tissue before and after using formalin and ethanol

Table 2 Odor of Tissue Before and After Preservation

Odor	Formalin's Preservative Formula	Ethanol's Preservative Formula	p-value (Chi-square Test)
Before			No statistic computed because is constant
Stink	32	32	
After			0.000
Pungent	32	0	
Pleasant	0	32	

Table 3 Consistency of Tissue Before and After Preservation

Consistency	Formalin's Preservative Formula	Ethanol's Preservative Formula	p-value (Chi-square Test)
Before			0.599
Soft	16	16	
Moderate	16	16	
After			0.599
Moderate	16	16	
Hard	16	16	

as a preservative formula had no significant difference because the p-value was more than 0.05. Thus, both preservative formulas had the same result in preserving the consistency of the tissue (Table 3).

Both preservative formulas were able to inhibit the growth of bacteria on tissues (Table 4).

Discussion

Better quality of tissue had been produced by using ethanol's preservative formula compared to formalin's preservative formula. Firstly, in terms of color, tissues that were preserved by ethanol's preservative formula were more similar to the tissues before preservation rather than formalin's preservative formula. Apparently in "Substitution of formaldehyde in cross anatomy is possible" by Hammer et al,8 tissues preserved by ethanol are better than tissues preserved by formalin, because tissues preserved by ethanol are easily distinguishable. Tissues that were preserved by formalin's preservative formula had grayish hue. This is because formaldehyde in formalin's preservative formula converts hemoglobin into methaemoglobin which is purple or black in color.[3] It will also cause the oxidation of ferrous iron which forms ferric oxide.[3] Therefore it gave the tissue a grayish appearance.[3] Hence, ethanol's preservative

Table 4 Growth of Bacteria on Tissue Before and After Preservation

Growth of Bacteria	Formalin's Preservative Formula	Ethanol's Preservative Formula
Before		
Positive	32	32
After		
Positive	0	0
Negative	32	32

formula showed better retention of color rather than formalin's preservative formula.

Secondly, in terms of odor, the tissues that were preserved by ethanol's preservative formula are pleasant because it contains high concentration of ethanol where its standard odor is pleasant.[9] However, the odor of tissue preserved by formalin's preservative formula is pungent because it contains formaldehyde where its standard odor is pungent or rather suffocating.[3,6,8]

However, both preservative formulas failed to retain the consistency of tissues. The reason of formalin's preservative formula causes hardening of tissue is that formalin cross-links the protein and stabilizes the mass of tissue.[3] On the other hand, ethanol's preservative formula also causes hardening of tissue. This is because ethanol precipitates the protein molecules of tissues.[10]

Both preservative formulas are able to inhibit the growth of bacteria on tissues. The reason of formalin's preservative formula being able to inhibit the growth of bacteria is that formaldehyde acts as bactericides, germicides, and fungicides.[1,4] This is because formaldehyde destroys the colloidal nature of molecule, and connects to amine group in protein molecules with nitrogen in a protein molecule by cross-linking.[3,10] This will fix the cellular protein and therefore cannot be a nutrient source for bacteria.[1] Besides, ethanol's preservative formula is also able to inhibit the growth of bacteria because it contains 70% ethanol which serves as antiseptic.[4] This is due to its bactericidal activity by denaturation of proteins.[10]

In conclusion, ethanol's preservative formula preserves better quality of tissue in color, odor and negative growth of bacteria.

The limitation of this study was its inability to preserve all organs of the study subjects due to time limits. Moreover, due to resource limitations, the method of humans killing laboratory animals can also be performed by administering Xylazine or Ketamine to reduce suffering of laboratory animals. Besides, due to human resource limitations, there were only two observers to access the quality of tissues. Apparently, the number of observers should increase to avoid bias. Finally, a further study is recommended by changing the amount of sodium chloride in both preservative formulas into smaller percentage to improve the consistency of the tissue preservation. Moreover, ethanol's preservative formula can be recommended to replace formalin's preservative formula to preserve cadavers for anatomy specimen due to lower health risk to the lecturers, technicians and students and its better quality of tissue preservation.

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