Effect Of Storage Conditions On The Morphological Preservation Of Rabbit Kidney And Liver Tissues

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Objective: To see the effect of different solutions and storage temperature on morphological preservation of rabbit kidney and liver tissues.

Material and Methods: In this experimental study, rabbit kidney and liver slices were stored in Ringer Lactate, Euro Collins and University of Wisconsin solutions (UW solution), for various fixed time intervals. The three solutions were tried at room temperature as well as at 0-4°C. Morphological preservation was assessed by a semi quantitative method.

Results: Adequate morphological preservation of both tissues was obtained in all solutions at room temperature for the first 24 hours. Lowering of storage temperature to 0-4°C was highly beneficial to both tissues in all solutions. UW solution is more effective for liver tissue preservation.

Conclusion: Anyone of the solutions may be used at room temperature, for preservation of tissue to be submitted for immuno-histochemistry for the first 24 hours. If prolonged storage is required, lowering of temperature is recommended.

Keywords: Organ preservation, Ringer lactate, Euro Collins solution, University of Wisconsin solution, morphological preservation, temperature.

Introduction

Tissue preservation is a major concern in histo-pathological studies.[1] Formalin has long been used as a "routine" tissue preservative and a fixative, but it has numerous drawbacks, the most troublesome of which is its masking of antigenic sites. This interferes with subsequent immuno-histochemical procedures. So the development of alternative preservation techniques is an ongoing challenge to the histopathologist.[3]

Fortunately histopathologists are not alone in their efforts to achieve tissue preservation. Transplant surgeons are also on a lookout for solutions which would give optimal organ preservation. This would reduce the constraints on time which could be allowed to elapse between organ procurement and its re-implantation.[4-6]

Ringer lactate, Euro Collins solution (EC solution) and University of Wisconsin solution (UW solution) are the most widely used organ preservation solutions.[4,5,7] Ringer lactate was amongst the earliest solutions tried.[8] Later, EC solution was developed which vastly improved the outlook for the kidney, but unfortunately did not benefit liver preservation.[9] After further extensive research, UW solution was developed at the University of Wisconsin. It was a complex multi component solution and provided markedly enhanced preservation of several organs especially liver and pancreas.[10]

Another factor found to be significant in tissue and organ storage was the storage temperature. Hypothermic preservation, short of freezing, gave promising results.[11,12]

In this study an attempt has been made to compare the morphological preservation of rabbit kidney and liver tissue in the three above mentioned solutions, both at room temperature and at 0-4°C. The aim was to study the effect of storage conditions on morphological preservation of these tissues and help to determine pragmatic choices made on grounds of cost and logistics.

Material and Methods

Rabbits were sacrificed and their kidneys as well as livers were sliced. These slices, 3-4 mm thick, were preserved in three different solutions at room temperature as well as in hypothermic conditions. The solutions were Ringer lactate, EC solution and UW solution. Ringer lactate was purchased from Medisol®, while EC solution was prepared after Abebe et al[13] and UW solution was prepared after Baatard et al[14] immediately prior to use. The storage temperature was kept around 25°C for the room temperature groups and at 0-4°C for the hypothermic groups **(Table 1).**

Tissue slices were removed at various fixed time intervals (0 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, and 96 hr) and processed routinely. Six tissue slices were

included in each group.
Sections were stained with haematoxylin and eosin. Morphological preservation was assessed by a semi quantitative method and expressed in percentage.[2] The 0 hr sections served as a control for each group. Observations were recorded by two independent observers. The results were then compared using paired student's t test and slope test.

Results

Satisfactory morphological preservation of both tissues was seen in all solutions at both temperatures for the first 24 hours. Thereafter, the temperature of 0-4° C gave a significantly better preservation. UW solution was found to give better liver preservation at both temperatures, compared with the other two solutions **(Table 2).**

Table-1: Details of storage conditions

Group	Solution	Temperature
A	Ringer Lactate	Room temperature
B	Ringer Lactate	0-4° C
C	EC Solution	Room temperature
D	EC Solution	0-4° C
E	UW Solution	Room temperature
F	UW Solution	0-4° C

Table-2: The effect of lowering of temperature on morphological preservation of rabbit kidney and liver tissues

Tissue	Group	Percentage presevation			Slope	p value
		At 24 hrs	At 48 hrs	At 96 hrs		
Kidney	A	65	45	19	-0.83	p=0.0000
	B	99	99	95	-0.04	
Liver	A	83	40	15	-0.97	P=0.0011
	B	93	83	65	-0.35	
Kidney	C	95	53	18	-0.98	P=0.0000
	D	97	93	88	-0.12	
Liver	C	85	40	15	-0.98	P=0.0018
	D	94	77	60	-0.42	
Kidney	E	94	70	64	-0.39	P=0.0312
	F	93	85	80	-0.21	
Liver	E	96	75	40	-0.61	P=0.0093
	F	93	80	65	-0.38	

Discussion

The current boundaries of tissue preservation have been set in an era of formalin fixation. The dire need of a better tissue preservation medium is obvious to anyone involved in immunohistochemistry and enzyme histochemistry.[3] Encouraging results were obtained in the current study.
The results showed that if the tissue to be subjected to immunohistochemistry can be transported to a referral centre within 24 hours, Ringer lactate at room temperature can give an adequate degree of preservation. Despite its simple composition Ringer lactate has shown acceptable degree of preservation in other studies.[15] If transport involves a longer duration of time, lowering the temperature to 0-4° C would be helpful in preserving morphology. Improved preservation of tissues at lower temperature is borne out by other studies.[16,17]
This happens because all enzyme activity is temperature dependent. Cooling diminishes metabolic activity, curtails oxygen demand and slows deterioration of energy stores. Also oxygen is more soluble at lower temperatures, allowing that dissolved in surrounding fluid to be utilized better.[18,19]
However, a few studies have suggested that a higher storage temperature of 10° C is better for preservation of endothelial cells.[20]
Another point that deserves mention is that we live in a hot country. Our environmental temperature may soar above 50° C. The temperature in our study was kept at 25° C. The environmental temperature must be kept in mind while deciding whether or not to use

this tool of hypothermia.

The results also show that liver tissue has an unmistakable preference for UW solution. This superiority of UW solution in liver preservation is well established.[21] With its components "designed" and "tailored" to cater to the need of the hepatocyte, UW solution remains a solution of choice for liver preservation. The only obstacles to its more widespread use are its high cost, reduced availability and limited shelf life.[22]

In conclusion, Ringer lactate at 0-4°C is the best option for renal tissue preservation. Liver tissue would fare better if stored in UW solution. The maintenance of a temperature of 0-4°C is an important tool in improving preservation of both tissues in all solutions.

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Endothelial cell preservation at 10 degrees C minimizes catalytic iron, oxidative stress and cold induced injury. Cell Transplant 2006; 15: 499-510.
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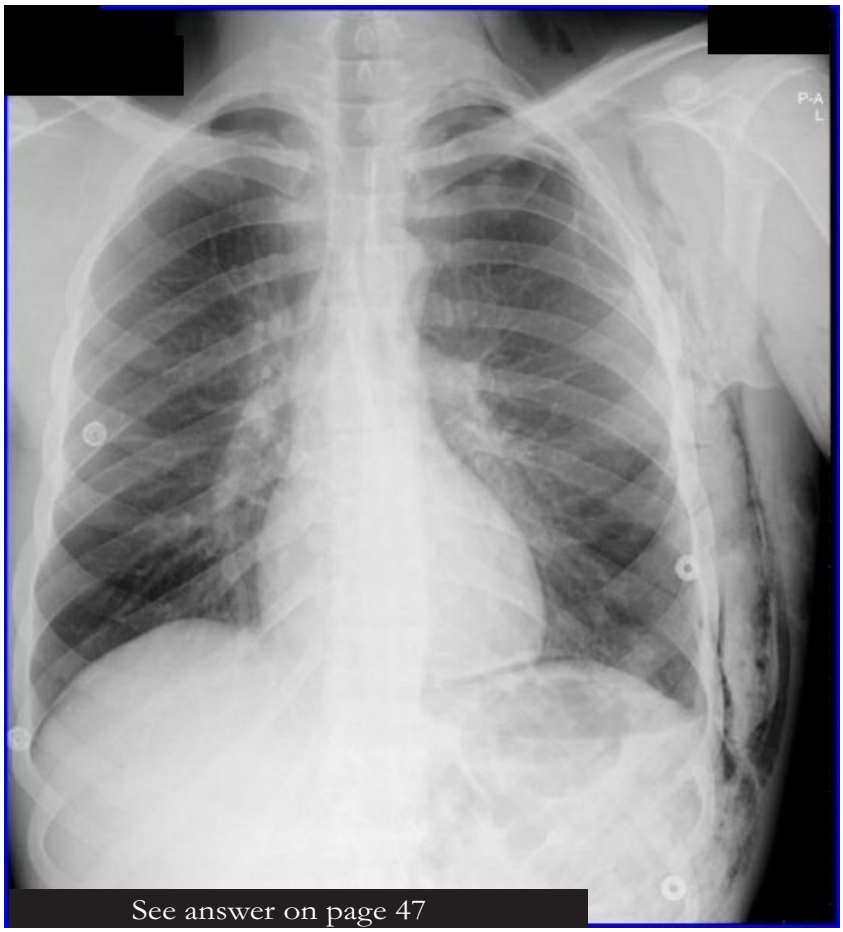
Picture Quiz

Please examine this x-ray carefully and answer following questions.

1. What abnormality do you see on this x-ray chest?
2. What physical findings may be present in this case?
3. What may be the complications?
4. How will you treat this patient?



See answer on page 47