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Evaluation for sterility of four crystalloid solutions used in preservation of harvested canine kidneys intended for transplant

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Abstract

This study investigated the sterility and Preservability of Ringer's Lactate, Darrows, 5% dextrose and Normal Saline solutions used as preservatives for 16 freshly harvested normal canine kidneys from 16 Nigerian local dogs of non specific sex and weight.

Nephrectomised kidneys were flushed and preserved for 24, 48, 72 and 96 hours at 4 0C in labeled bowls containing heparinized antibiotics incorporated in the solutions with each having its control (without antibiotic penicillin/streptomycin incorporated). Pre and post preservation, a loop-full of each solution was cultured on freshly prepared two general purpose media (Blood and nutrient agar) and one selective medium (MacConkey agar). They were incubated at 37 0C for 48 hours after which they were examined for bacterial growth. Histological studies of the preserved kidneys were used to predict preservability.

There was no evidence of microbial growth upon 96 hours of culture for all the solutions their respective controls). Generally, the gross appearances of the kidneys were normal indicative of viability.

In conclusion, the indigenous, commercially produced intravenous fluids that were used for preservation of harvested kidneys were sterile before and after 96 hours of preservation. This was evident by the absence of microbial growth in both groups of the solutions after 96 hours preservation.

Keywords: Sterility, Solution, Canine Kidney, Preservability, Transplantation.

Introduction

Several solution have been used for preservation of harvested kidneys, these include: Collins, Modified Collins, Sacks, Hyperosmolar citrate, University of Wisconsin (UW), Modified University of Wisconsin, Perfudex, Chlorpromazine, Cryoprecipitated Plasma, Gaseous Oxygen Perfusion (adjunct in kidney preservation) and Euro-Collins solutions. Ploeg *et al.* (1988) reported 72 hours cold storage of kidney with UW. Ploeg *et al.* (1990) recommend UW solution as an effective preservative and flush solution for all intra-abdominal organs used for transplantation. Lin *et al.*, (1995) used UW to preserve the liver and the pancreas. Although the University of Wisconsin (UW) was considered the standard solution for the preservation of kidneys for transplantation, the colloid hydroxyethylstarch (HES) contained therein has been shown to be controversial (Schlumpf *et al.* 1995). Yin *et al.* (1996) improved preservation time of UW solution by addition of retrograde oxygen persufflation. This will ameliorate energy loss and improve functional recovery of ischaemically-injured kidneys. Candinas *et al.* (1996) used Dextran 40 based preservation solution, which substituted HES in UW solution but showed no overall improvement in graft survival rates. Rabbit kidneys preserved in Euro-Collins alone did not improve preservation time nor did it protect vascular endothelium, which regulates function and urine excretion in comparism with Euro-Collins plus Thromboxane A2 inhibitors (UK 38485), (Kuzu *et al.*, 1995). However Kumada *et al.* (1997) used Ouabain containing Euro-Collins solution to preserve kidneys. He found that it protects proximal tubular cell against ischaemic damage. Collins solution is one of the world's widely used solutions for kidney preservation (Kreis *et al.*, 1978). For preservation to be successful, the preservative must be sterile as a surgical requirement for asepsis. None of the several solutions used with various success levels, have been reported of being contaminated with pathogens. This study therefore was aimed at investigating the sterility of our indigenous solutions used as preservatives for canine kidneys intended for transplantation.

Experimental design and Methods

Sixteen kidneys were harvested for evaluation from sixteen adult Nigerian local dogs of none specific weights and sexes. The dogs were conditioned and acclimatized for 2 weeks before the study. The dogs were judged to be clinically healthy after presurgical evaluation by physical examination and laboratory evaluation with haematology, serum chemistry and urinalysis. These parameters were taken two times a week (n=5) for all dogs under study. Temperature values were taken on daily basis for the 14 days pre- and post-surgery. Food and water were withheld for 3 hours before all the surgeries and all dogs underwent aseptical preparation prior to surgery with liberal clipping of the ventral abdomen and scrubbing with 2 % chlorhexidine solution (Purit® Saro LifeCare Nigeria).

Anesthesia: For general anesthesia, thiopental sodium (20 mg/kg intravenously) was the main anaesthetic agent used in continuous infusion after premedication with atropine (0.05 mg/kg intravenously) and chlorpromazine (4 mg/kg intravenously). Post anaesthetic induction, all dogs were intubated, placed on dorsal recumbency and draped aseptically.

Surgical technique and approach: A 12 cm Cranioventral linea alba incisions was made 1cm from the xiphoid and extending 1 cm to the umbilicus, to access the kidneys at the retroperitoneal space. The vessels (renal arteries first and then renal veins) and ureters were adequately identified and ligated with 3-0, chromic catgut and severed. Abdominal closures were routine in 3 layers (Peritoneum and Linea alba; and subcutis with 2-0 chromic catgut and the skin with 2-0 nylon.

Following harvesting, two kidneys were placed in eight small bowls labeled Ai and Aii, Bi and Bii, Ci and Cii and Di and Dii containing 500 ml of indigenous, commercially available electrolyte flush/storage solutions (Ringers Lactate –Ai; Darrows–Bi; 5% Dextrose-Ci and Normal Saline-Di) in which 15000IU of heparin, 5ml of 2 % xylocaine, 400000IU penicillin and 75mg streptomycin were incorporated and their respective controls (Aii, Bii, Cii and Dii) without antibiotics. From these solutions, test samples were collected for microbiology culture. These solutions were used to flush the kidneys using 20mls sterile syringes and cannulae. Post flushing, the kidneys were preserved in the same solutions, chilled at 4 ºC in the refrigerator for 0-24, 0-48, 0-72, 0-96 hours respectively. Upon expiration of each storage time, all kidneys were grossly examined and then fixed in buffered formalin for histological study.

Microbial study

Upon termination of storage, samples for microbiology studies were again collected. A loop-full of each of the solution was cultured on freshly prepared two general purpose media (Blood and nutrient agar) and one selective medium (MacConkey agar). These media were prepared by mixing the powders in distilled water following the manufacturer's instructions. These were autoclaved and poured aseptically into clean sterile Petri dishes and allowed to solidify. A loop-full each of solutions (pre-preservation and post preservation) Ai and Aii, Bi and Bii, Ci and Cii and Di and Dii were streaked unto these freshly prepared media and incubated at 37 0C for 48 hours after which they were examined for bacterial growth.

Results

All pre-surgical evaluations (temperature, haematological and serum chemistry values) were normal. Grossly, the preserved kidneys were normal in comparison with freshly harvested ones, the kidneys appeared bright and blanched. Histology revealed normal sections, well preserved renal architecture though slight tissue swellings were noticed for kidneys preserved in some solutions for 96 hours. Cortical and medullary sections showed normal histology for the kidneys preserved for up to 72 hours in all solutions.

There was no growth in all cases (Plates 1a & b).

Plate 1a No growth after 48 hours culture at 37 0C in Blood, Nutrient and MacConkey agars

Plates showed no evidence of microbial growth from preservative solutions cultured after 24 hours organ storage

Plate 1b No growth after 48 hours culture at 37 0C in Blood and nutrient agars

Plates showed no evidence of microbial growth from preservative solutions after 96 hours organ storage

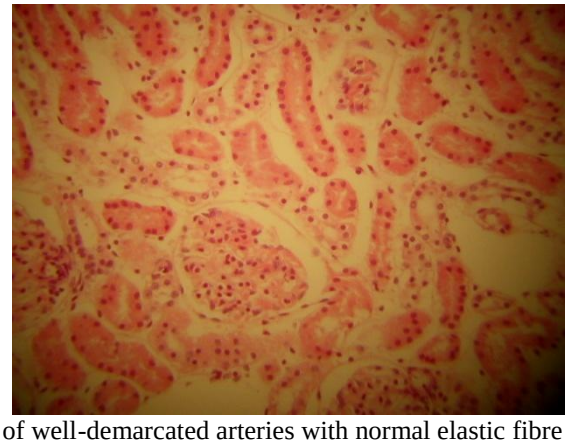

Plate 2 Scanned photomicrographs of well-demarcated arteries with normal elastic fibre endothelium. Glomeruli-G well

preserved, capillary turfs-C evenly spread out. Urinary space Us adequate and empty, Vascular pole patent. Mesengial cells appear neat with granular cytoplasm at 96 hours X80

Discussion

Kidney harvesting and preservation have been reported widely with variable successes. Preservability of harvested kidneys in the studied electrolyte solutions at 4 °C for up to 96 hours is a significant contribution towards attaining the desired long-term preservation without contamination. Absence of growth after 48 hours culture (Plates 1a & 1b) indicated that all the solutions were sterile and also there were no contaminations at all levels of the study. The antibiotics incorporated in the control served no extra purpose but could be incorporated in situations where fears are nursed on the aseptical protocols in the entire process of harvesting and preservation or where there are doubts as to the sterility of the storage solutions.

The histology of the kidneys was normal with the glomeruli and the tubules appearing well preserved, an evidence of absence of pathogen invasion. Minimal changes of the renal parenchyma were insignificant to distort the normal physiological functioning of the organs if grafted. These changes were largely due to fluid infiltration into the tissues as a result of electrolyte imbalance (i.e. low electrolyte concentrations) and these were insignificant compared to those reported by Barry *et al.* (1981) in acceptable preservations. Also the results of 96 hours preservation implies a more successful preservation than the 48 hours recorded in Collins solution (Collins *et al.*, 1969; Collste *et al.* 1970).

These results showed that it is possible to harvest kidneys intended for transplant and preserve in any of our indigenous, commercially prepared electrolyte solutions at 4 °C for up to 96 hours without fear of microbial invasion.

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