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Effect of Perfusate Osmolarity on Hearts Preserved Under Hypothermic Pulsatile Perfusion for 24 Hours

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Canine hearts preserved under hypothermic pulsatile perfusion (24 hours) with an osmolarity of 340 mOsm / L had better preservation and transplantation response than hearts perfused at an osmolarity of 310 and 270 mOsm / L. The hearts perfused at 340 mOsm / L survived an average of 22.6 days after transplantation. This survival was significantly ($p>0.05$) better than that observed after fresh transplantation (13.4 days average). Hearts perfused at 270 mOsm / L had a significantly ($p>0.05$) lower survival after transplantation (5.8 days average) than the fresh allografts or the hearts perfused at 310 or 340 mOsm / L. Similar survival times were observed for the fresh transplants and those perfused at an osmolarity of 310 mOsm / L (17.8 days average). Thus, osmolarity appears to be an essential factor for hypothermic pulsatile perfusion of hearts.

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Introduction

A number of factors, such as perfusion pressure, osmolarity, temperature, and type of perfusion are important in heart preservation. In a recent study, we described the effect of systolic perfusion pressure on canine hearts under hypothermic pulsatile perfusion for 24 hours.[1] In this paper, we report the results of different degrees of osmolarity on canine hearts preserved under hypothermic pulsatile perfusion for 24 hours.

Material and Methods

Adult mongrel dogs of either sex, weighing from 9 to 25 kg, were anesthetized with sodium methohexital for induction and halothane for maintenance (0.5% - 2.0%). During anesthesia the animal received oxygen (1 L / min) through an endotracheal tube connected to a volume respirator. Two hundred and fifty to 500 ml of Ringer's lactate were given intravenously during surgery. Through a median sternotomy the heart and great vessels of the donor animal were dissected, the aortic arch branches were tied off and divided, and the heart was then excised and flushed out through the aorta with 500 ml of Ringer's lactate solution containing 10,000 units per liter of heparin at 4°C. Flushing pressure was 40 cm of water; flushing time was that required for the Ringer's lactate solution to be administered. The heart was then placed in a hypothermic pulsatile perfusion machine (Mox-100)* for 24 hours, and was perfused through the aorta or the subclavian artery. The perfusion conditions were hypothermia (7°C), pO_2 200 mm Hg, pCO_2 adjusted to maintain a pH of 7.35, and a systolic perfusion pressure of 25 mm Hg. The perfusate basically consisted of doubly frozen, thawed and filtered cryoprecipitated plasma (CPP). The plasma had the following additives per liter: magnesium sulfate 8 mEq, PSP dye 2 ml, insulin 80 units, penicillin 250,000 units, mannitol 5-15 gms, and methylprednisolone 250 mg. The variables in the perfusate were determined by the amount of albumin and potassium added. The os-

*Waters Instruments, Rochester, Minnesota.

molarity varied according to the amount of the previous substances added. During perfusion, the superior and inferior vena cavae, the pulmonary artery, and the pulmonary veins were left open for free drainage (Figure 1).

The heart was weighed before and after perfusion. Sodium, potassium, and lactic acid were determined in the perfusate at 0, 1, 2, 3, 6, 12, and 24 hours.

After perfusion, the heart was transplanted into the abdominal vessels of the recipient dog. The abdominal aorta and the inferior vena cava were exposed through a midline laparotomy. The aorta of the donor heart was sutured to the recipient's abdominal aorta with 5-0 prolene suture, and the pulmonary artery was sutured to the recipient's inferior vena cava with a 5-0 prolene suture. Prior to the vascular anastomosis, the donor aortas and the pulmonary arteries were trimmed to 1.0 cm above the valves to avoid any outflow obstruction. The superior and inferior vena cavae from the donor hearts were stick tied with 2-0 tevdek. The vascular clamps were released, any air was removed from the left ventricle or aorta, the heart was gently massaged, and 1 cc of 10% calcium chloride and / or epinephrine 1:10,000 were injected into the left ventricle. If the heart did not start spontaneously after 2½ to 5 minutes of direct massage, it was defibrillated with electric countershock.

Full thickness samples of the left ventricle (always from the same site and at the same experimental step) were obtained for light microscopy after perfusion, upon transplantation, and before death. Most of the samples were evaluated at the end of preservation and before transplantation in order to avoid the rejection changes observed in this transplant model. Multiple sections of each sample were examined. Samples of histology were fixed in 10% buffered formalin.

All recipient animals received Ringer's lactate 1000 ml in 24 hours for two days. Thereafter, the diet was restarted gradually. All the animals also received azathioprine 5 mg / K per day for the first three days and then 2.5 mg / K per day until death. Ampicillin 1 gm IV or IM was given daily for three days.

All dogs were clinically assessed every day. Strength of the heartbeat was determined by palpation. Electrocardiograms

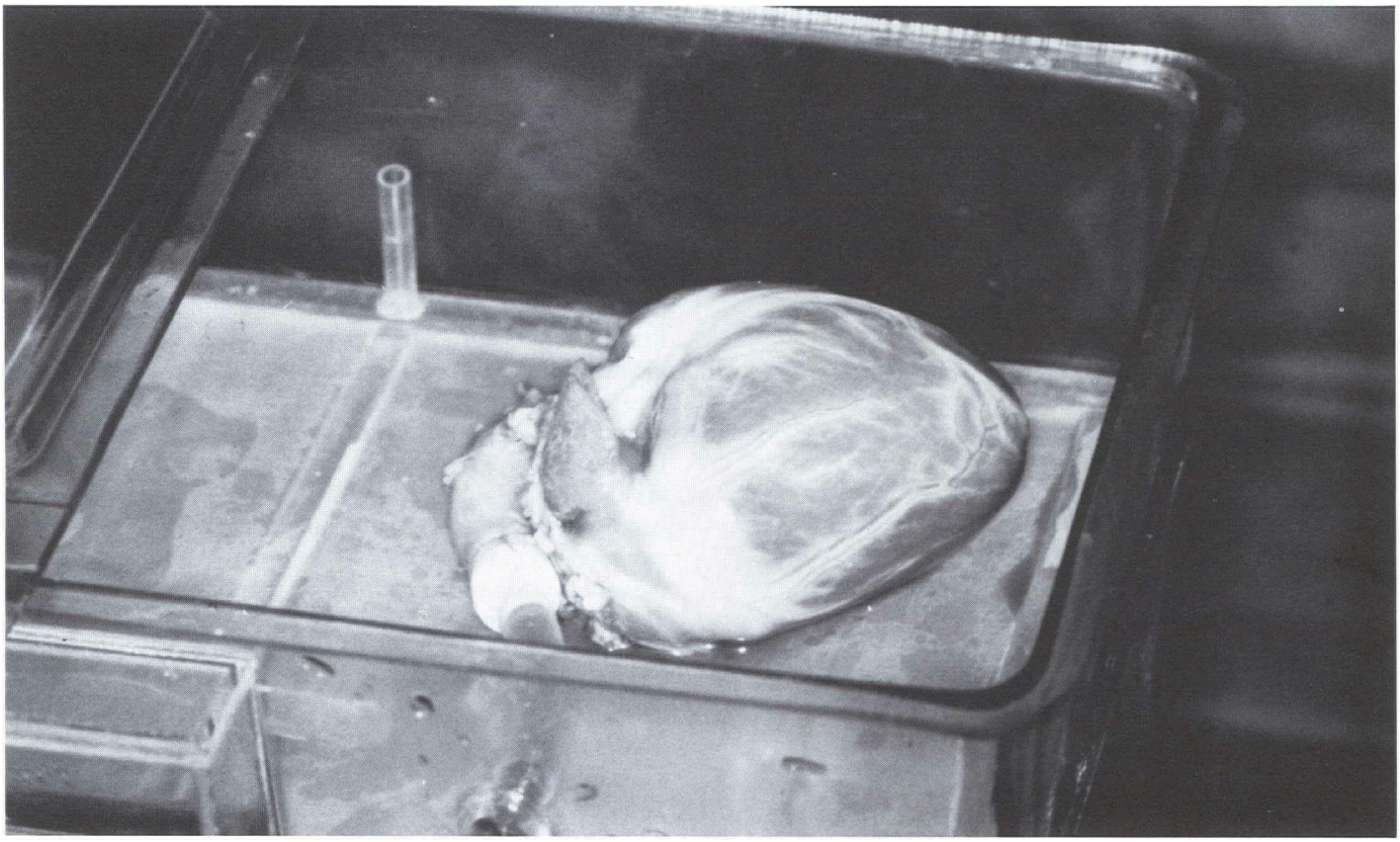

Fig. 1

Canine heart preserved under hypothermic pulsatile perfusion (Mox-100, Waters Instruments Co). The perfusate goes through the aorta and drains into the coronary sinus and eventually the right side of the heart. Both vena cavae and pulmonary veins are left open for further drainage.

(ECG) were taken immediately after surgery and every other day until the end of the study. When no heartbeat was identified and no electrical activity found in the ECG, the heart was removed for final study. Postmortem examination was performed on all dogs.

Four groups of recipient animals (five dogs per group) were included in this study: Group I, control, fresh transplanted hearts without perfusion; Group II, hearts perfused with CPP without albumin or potassium added (osmolarity: 270 mOsm/L); Group III, hearts perfused with CPP that had albumin (12.5 gm/L) and dextrose 50% (5 ml/L) (220 mg%) added (osmolarity: 310 mOsm/L); and Group IV, hearts perfused with CPP that had the same albumin as Group III, in addition to dextrose 50% (10 ml/L) (420 mg%) and potassium chloride (final concentration, 50 mEq/L) (osmolarity:340 mOsm/L).

Five dogs were eliminated from the final groups for technical or unrelated causes of death, such as distemper in one dog (Group I), intussusception in another (Group IV), aortic or pulmonary artery bleeding in two (Group I and II), and anesthetic overdose in one (Group II). Statistical analysis with standard error and Student's t-test were used for all parameters of the four groups.

Results

Hearts perfused at an osmolarity of 270 mOsm/L had 12.0 ± 2.7% (M ± SE) weight gain during the first hour of perfusion. Thereafter, there was a gradual and progressive weight gain until the end of the study (Figure 2). The systolic perfusion pressure increased during the first three hours of perfusion (5.0 ± 2.0 mm Hg) (M ± SE), after which there were no significant changes ($p>0.1$). The perfusate flow decreased from 1.0 ± 0.15 ml/min/gm (M ± SE) to 0.9 ± 0.1 ml/min/gm (M ± SE) in the first two hours of perfusion. The flow remained stable for the following four hours and subsequently decreased to 0.8 ± 0.1 ml/min/gm (M ± SE) at 12 hours. Thereafter, it remained constant. No significant changes were noted in the perfusate sodium, potassium, or lactic acid at any time during the study.

Hearts perfused at an osmolarity of 310 mOsm/L had moderate weight gain during the first six hours of perfusion (11.5 ± 3.2%) (M ± SE). Thereafter, there was a minimal weight gain ($p>0.5$) at 12 and 24 hours (Figure 2). The perfusate pressure remained stable at the initial determination for the first three hours, after which there was a minimal decrease at six hours (20 ± 4.5 mm Hg) (M ± SE). Thereafter, it remained at this level until the end of the study. The perfusate flow showed practically no changes during the whole perfusion period (1.1 ± 0.1 ml/min/gm) (M ± SE). No significant changes were observed in the perfusate sodium, potassium, or lactic acid at any determination during the perfusion time.

Hearts perfused at an osmolarity of 340 mOsm/L had no significant weight gain throughout the whole study. The maximal weight gain at 24 hours was 7.5±2.7% (M±SE) (Figure 2). The systolic perfusate pressure (25 mm Hg) and perfusate flow (1.2ml/min/gm) showed no significant changes during the whole perfusion period. No chemical perfusate changes were observed throughout the whole study.

Four hearts perfused at an osmolarity of 270 mOsm/L required resuscitation to start regular contractions. Only one heart perfused at 270 mOsm/L beat spontaneously after the administration of Ca Cl^2 and epinephrine. These grafts survived an average of four days (Figure 3). Two hearts

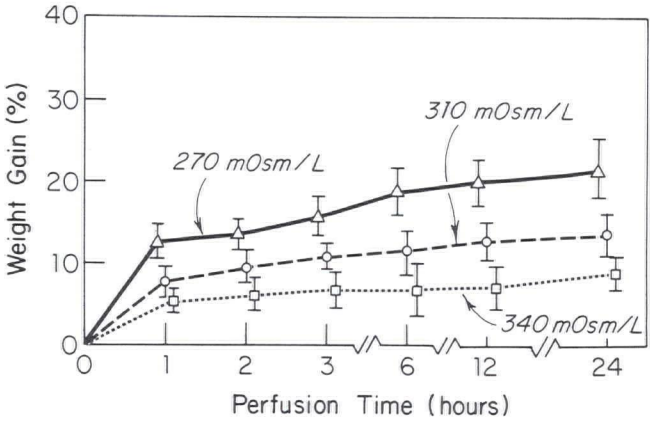

Fig. 2

Effect of perfusate osmolarity on weight gain during perfusion. Less weight gain was observed in the hearts perfused with the highest osmolarity (340 mOsm/L). In contrast, considerable weight gain was noted in the hearts perfused at low osmolarity (270 mOsm/L).

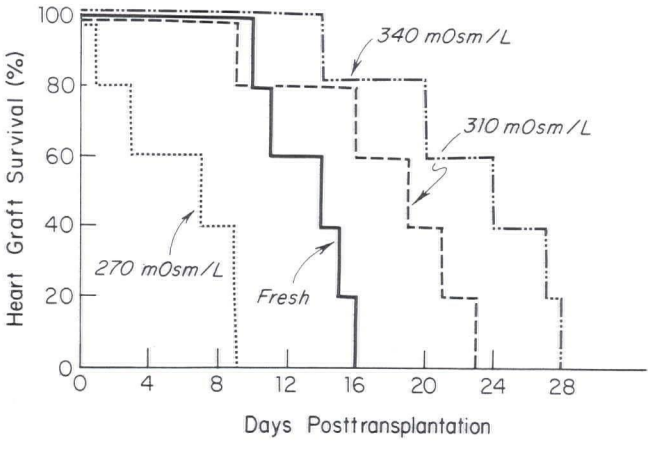

Fig. 3

Heart survival after heterotopic transplantation. Graft survival was directly related to the perfusate osmolarity: the higher the osmolarity, the longer the survival observed.

perfused at an osmolarity of 310 mOsm/L beat spontaneously after the administration of $CaCl^2$ and/or epinephrine, but the remaining three hearts required chemical and electrical resuscitation. The average survival time for these grafts was similar to that of the fresh heart allografts ($p>0.5$) (Figure 3). If the osmolarity in the perfusate was increased to 340 mOsm/L, there was an even better response in graft function and survival (Figure 3). Hearts perfused at 340 mOsm/L for 24 hours had significantly ($p>0.05$) better survival after transplantation than the fresh allografts. After transplantation, three hearts had spontaneous contractions, but the two remaining hearts responded only after chemical and electrical defibrillation treatment.

Hearts perfused at 340 mOsm/L had no histological damage after perfusion (Figure 4), while those perfused at lower perfusate osmolarity (270 and 310 mOsm/L) showed minimal to moderate edema and occasional banding. Two hearts perfused at 270 mOsm/L showed severe ischemic changes.

Discussion

This study demonstrates that hearts perfused with high osmolarity (340 mOsm/L) had longer preservation times and better survival response after transplantation than the hearts perfused at lower osmolarity (310 or 270 mOsm/L). There was a direct relationship between the level of osmolarity and heart viability during perservation and after transplantation.

In addition to confirming the reports of other investigators,[2,3] our work defines in a systematic way the ideal osmolarity for hypothermic pulsatile perfusion of canine hearts. Levitsky and his group[2,3] demonstrated that hyperosmolarity of a colloid perfusate (CPP) was an important factor during heart preservation. Hearts perfused with a high osmolarity solution had good myocardial contraction, decreased compliance, and improved left ventricular work. They did not, however, transplant the hearts after physiological evaluation. Copeland et al[4] do not believe that the increase in oncotic pressure plays a significant role in heart preservation. However, their system was a nonpulsatile one that used dextran as the oncotic agent. The high osmolarity of our perfusate was based mainly on the use of albumin and intracellular electrolyte composition.

Fig. 4

Histology of a preserved heart under hypothermic pulsatile perfusion for 24 hours with 340 mOsm/L in the perfusate (HEX 450). There is no evidence of any structural damage. Cellular elements are well preserved with no ischemic injury.

We do not have a clear explanation for the better results of hearts perfused with an hyperosmolar colloid solution. It is possible that edema is prevented because high osmolarity decreases the movement of fluid into the extracellular space. Also, as mentioned before, our plasma preparation basically used salt-poor albumin and potassium to increase osmolarity, and these two substances in themselves are sufficient to improve preservation. The albumin could draw some interstitial fluid into the intravascular space and therefore diminish the fluid in the extravascular space. Potassium, on the other hand, could stabilize cations and prevent the flux of these ions into the extracellular space.

Other factors besides osmolarity are important for heart preservation, such as perfusion pressure,[1,4–6] temperature,[4,7] and type of perfusion flow.[4,5,8,9] The use of hyperbaric oxygenation[10–12] has been replaced by current preservation methods. Others[13,14] have even indicated that a derangement in the ATPase system could also be an important factor for unsuccessful heart preservation.

Summary

The data presented in this report indicate that high osmolarity (340 mOsm / L) is beneficial for hypothermic pulsatile perfusion of canine hearts. Hearts perfused at an osmolarity of 270 and 310 mOsm / L did not perform as well as those perfused with a higher osmolarity solution. Furthermore, hearts perfused at high osmolarity lived significantly longer than fresh (unperfused) grafts. Whether, in view of this finding, perfusion could modify the immunogenicity of the graft in any way and therefore improve long-term survival is still open to question and further investigation.

Acknowledgment

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