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Prolonging Kidney Graft Survival with Concanavalin A: Effects of temperature, perfusate composition, pH, and different manufacturing lots

Luis H. Toledo-Pereyra, MD, PhD* and Richard L. Simmons, MD**

This study analyzes the effect of temperature, perfusate composition, pH, and variable manufacturing lots in prolonging kidney allograft survival with Concanavalin A (Con A). Cold temperature (4°C), crystalloid composition of the perfusates, and neutral or mildly alkaline pH were important factors in the effect of Con A on prolonging allograft survival. Also, different lots of Con A from the same manufacturer produced variable results in prolonging survival. Thus, multiple factors should be considered if Con A is to be used to prolong kidney allograft survival.

PROLONGED allograft survival has been achieved mainly by suppression of the recipient's immune response. Few studies have been concerned with prolonging graft survival by inducing modification of the antigenicity or immunogenicity of allografts. Treatment of allografts *in vitro* with corticoids,^{1,2} thalidomide,³ urethane,⁴ radiation,⁵ or electrophoresis^{2,7} have prolonged the survival of some allografts to a greater degree than systemic administration of these same agents. *In vitro* treatment of grafts with streptokinase-streptodornase,⁸ ribonucleic acid,⁹ or with purified recipient transplantation antigens¹⁰ does not prolong survival as well as pharmacological manipulations. Minimal or no immune reaction has been observed toward allografts treated *in vitro* with formalin, cyanide¹³ or by freeze drying.¹⁴ Even these efforts to alter allograft immunogenicity have used test systems involving normal, unmodified recipients. Such experimental systems are probably capable of detecting only the most profound alterations in graft immunogenicity, some of which may even lead to loss of graft viability.^{15,16}

We have developed a standard system for minimal immunosuppression in dogs which itself is insufficient to prolong renal allografts. But, in the modified recipient, prolongation of graft survival by *in vitro* manipulation can readily be observed. For example, modification of renal allograft by perfusion *in vitro* with heterologous antilymphocyte globulin, dipyridamole, phytohemagglutinin, or with Concanavalin A (Con A) will significantly prolong graft function and life in minimally immunosuppressed dogs.¹⁷⁻¹⁹

Our best results have been obtained when dog kidney allografts were perfused *in vitro* at 4°C with 25 mg/L of Con A before transplantation. While ultimate rejection always occurred, dog allografts occasionally survived for as long as 60 days. The doses of azathioprine used were not sufficient to produce prolonged survival of the kidney graft by itself. Similarly, perfusion of kidneys with optimal concentration of Con A had no effect on dogs who did not receive some systemic immunosuppression.¹⁹

In our initial experiments, perfusion of canine kidneys with Con A did not prolong the survival of transplanted dogs if the

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perfusion *in vitro* was performed at 25°C rather than at 4°C.¹⁹ We hypothesized that Con A became clustered on the fluid membrane of the graft endothelium at the warmer temperature¹⁹⁻²¹ so that antigen masking was incomplete. In contrast, at 4°C the fluid cell membrane may have been in a gel-like state,¹⁹⁻²⁰ which would allow the Con A to become more evenly diffused over the entire surface, thereby masking the evenly dispersed histocompatibility antigens. We further hypothesized that by placing the graft suddenly into the blood stream at 37°C immediately after transplantation, the proteins of blood became bound to the excess evenly distributed Con A on the endothelial membrane so that the antigen-masking effect was augmented.¹⁹ The present studies are an unsuccessful attempt to verify these hypotheses by experimental testing.

Our work also investigates other important factors in prolonging kidney allograft survival with Con A, such as pH of the solution and the manufacturing lot of Con A used. This last factor is of considerable importance, particularly in view of recent findings on the rat model,²² which were not able to reproduce earlier results in prolonging kidney allograft survival with Con A.²³

Material and Methods

Experiment 1

Forty-two adult mongrel dogs of either sex were randomly divided into seven groups of six dogs each. The dogs were nephrectomized and given heterotopically unrelated kidneys by standard operative techniques.^{17,19} After transplantation, 20 mg of furosemide were given intravenously, and all recipient dogs were given azathioprine 5.0 mg/kg/day intravenously for three days, then 2.5 mg/kg/day orally until death. Serum creatinine was recorded daily. The onset of rejection was defined as the day on which the creatinine was elevated above 2.0 mg%.

Immediately after donor nephrectomy, the kidneys were flushed from a height of four feet (120 cm/water pressure) with 500 ml of Ringer's lactate solution (pH 6.5, 4°C, procaine 0.1 gm/L, heparin 10,000 U/L). The kidneys of Group I were controlled without Con A in the perfusate, while those in Group II were flushed with Ringer's and Con A* (25 g/ml). The kidneys in Group III were flushed at 4°C with 500 ml of regular canine, normal pooled plasma containing 25 mg/L of Con A but neither procaine nor heparin. The kidneys from Groups IV to VII were first perfused with Ringer's lactate (500 ml) and Con A (25 mg/L), and then a second time with 50 ml of either plasma at 4°C (Group IV), Ringer's lactate at 4°C (Group V), Ringer's lactate at 37°C (Group VI), or plasma at 37°C (Group VII).

* Lot 210073, Cal Biochem Labs, San Diego, CA

Excluded from analysis were five dogs that died in the first three days after transplantation from distemper, postoperative hemorrhage, or anesthetic complications.

Experiment 2

Twenty-two mongrel dogs were divided into four groups: Group VIII (n=5) without Con A in the perfusate, and Groups IX (n=5), X (n=6), and XI (n=6) with 25 mg/L of Con A (Lot 210073 Cal Biochem Labs, San Diego, CA) in the perfusate, maintained at pH 5.6, 6.5, and 7.6, respectively. The pH was raised from 6.5 to 7.6 in the Ringer's solution with NaHCO₃ 0.1N, and HCl 0.1N was used to obtain a pH of 5.6.

Experiment 3

One more set of twenty mongrel dogs was divided into four groups of five dogs each: Group XII as the control without Con A in the perfusate; Group XIII transplanted with kidneys flushed with Con A, 25 mg/L (Lot 330307-NaCl, Cal Biochem Labs); Group XIV with the same concentration of Con A but a different lot (Lot 610049-NaCl, Cal Biochem Labs), and Group XV using kidneys flushed with purified Con A (Lot 610049-NaCl, Fraction II*).

Results

The survival of the recipients of all renal allografts is summarized in Figures 1-5. Dogs given normal kidneys rejected them in two weeks despite being immunosuppressed with azathioprine. Dogs transplanted with kidneys perfused with Con A in Ringer's lactate at 4°C survived for prolonged periods, while those which received Con A in plasma at 4°C did not (Figure 1).

In Groups IV-VII the kidneys treated at 4°C with Con A were immediately reperfused with 50 ml of plasma or Ringer's lactate. The prolonged survival of kidneys perfused at 4°C with Con A was negated by plasma perfused at 4°C or 37°C (Figure 2). Similar negation of prolonged survival of the altered kidneys occurred when 50 ml of Ringer's solution at 37°C were passed through the Con A-treated kidney (Figure 3).

Figure 4 shows the effect of pH on prolonging the survival of kidneys pretreated with Con A. The best results were obtained at pH 7.6. Figure 5 shows the effect of various lots of Con A in prolonging graft survival. While the original lots 330307 and 610049 had no significant effect on prolonging kidney survival, the purified Con A (Fraction II) did significantly affect the prolongation of kidney survival.

* Fraction II supplied by Cal Biochem Labs (Dr. M.E. Shantz). It was obtained by passing Con A through a Sephadex-G 50 column and dialyzing it several times against glucose and NaCl.

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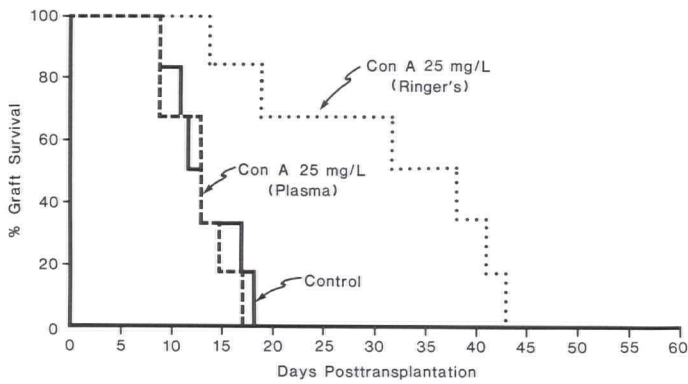


Fig. 1

Cumulative survival of canine kidney allografts perfused *in vitro* with Concanavalin A (Con A) in Ringer's lactate or regular dog plasma. These data include Groups I, II, and III.

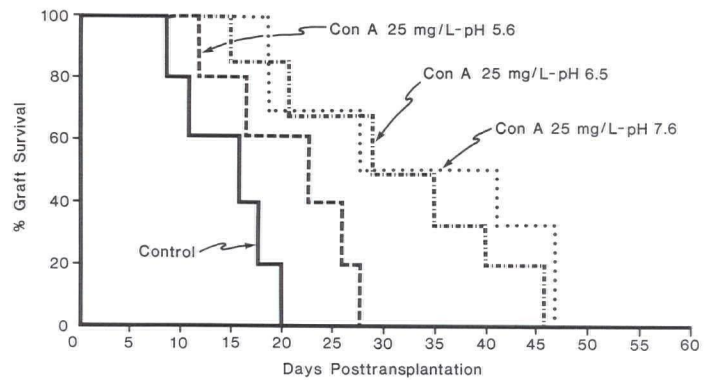


Fig. 4

Cumulative survival of canine kidney allografts perfused *in vitro* with Concanavalin A (Con A) in Ringer's lactate (4°C) at different pH levels. These data include Groups VIII, IX, X, XI.

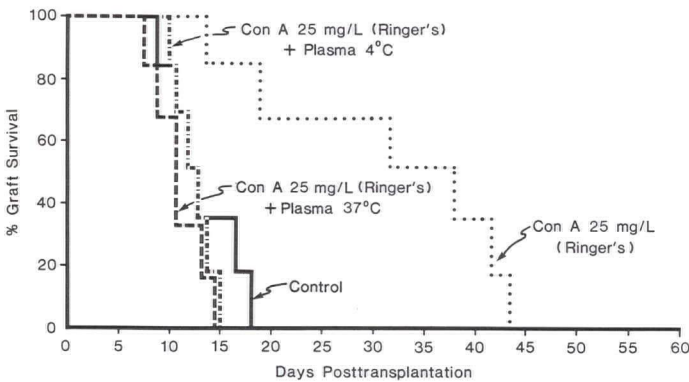


Fig. 2

Cumulative survival of canine kidney allografts perfused *in vitro* with Concanavalin A (Con A) in Ringer's lactate and reperfused with regular dog plasma at 4°C or 37°C. These data include Groups I, II, IV, and VII.

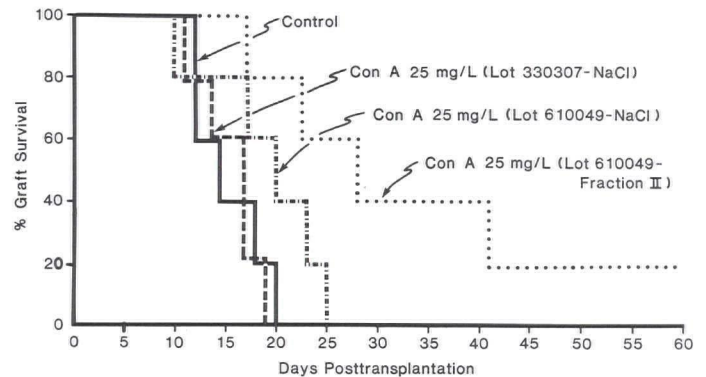


Fig. 5

Cumulative survival of canine kidney allografts perfused *in vitro* with Concanavalin A (Con A) in Ringer's lactate at 4°C. The effect of various lots of Con A was tested. These data include Groups XII, XIII, XIV, XV.

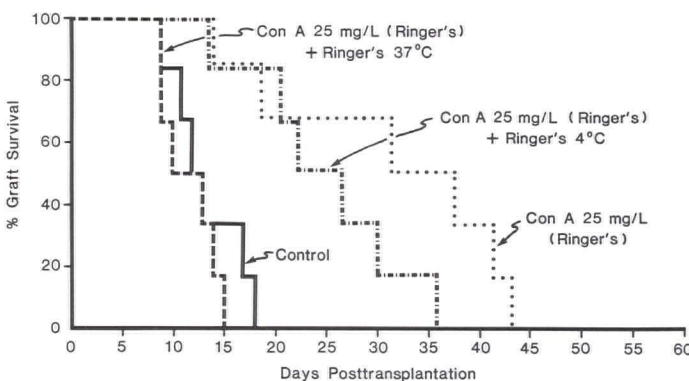


Fig. 3

Cumulative survival of canine kidney allografts perfused *in vitro* with Concanavalin A (Con A) in Ringer's lactate and reperfused with Ringer's at 4°C or 37°C. These data include Groups I, II, V, and VI.

Discussion

These experiments confirm our previous findings that canine renal allograft survival can be prolonged in minimally immunosuppressed recipients if the kidney is perfused with Con A in Ringer's lactate at 4°C.¹⁹ On the other hand, perfusion of kidneys with Con A in plasma at 4°C did not prolong the survival of dog kidney allografts. Plasma perfusion of the kidney even after modification with Con A in Ringer's solution at 4°C totally negated the prolongation. It is likely that the glycoproteins in plasma remove the Con A from its exposed position on the endothelium of the grafted kidney. In fact, perfusion with Ringer's solution at 37°C and to a lesser degree at 4°C reduces the graft modification initially obtained by perfusion with Con A at 4°C. These results shed some light on the mechanism by which Con A modifies the immunogenicity of renal allografts. Modification is obviously temperature dependent and can be

achieved only in the cold, although the exact optimal temperature is still unknown. The Con A is only loosely bound and can be effectively removed by plasma, presumably because of the high concentration of competitive Con A-binding glycoproteins in plasma. It can even be partially removed by small volumes of Ringer's perfusate at 4°C, although it is much more easily removed at the warmer temperature. It was previously considered that Con A, which is itself an immunosuppressive agent, acts synergistically with azathioprine to systemically immunosuppress the recipient dogs. We found, however, that systemic administration of Con A with azathioprine could not prolong the survival of unmodified kidney allografts.¹⁹

Nevertheless, a serious question remains. How is it possible for Con A to effectively modify graft immunogenicity at the same temperatures *in vivo* that remove it most easily *in vitro*? One possibility is that Con A on graft endothelium activates immunologically competent cells that flow through the graft in the first few minutes after grafting. Since it is known²⁴ that Con A can transform T-cells non-specifically, it is possible that it retains this mitogenic potential when it binds to cell membranes. Moreover, the known capacity of Con A to stimulate suppressive T-cells may in some way be augmented by attachment to the cell membrane.¹²

Our studies also indicate that Con A in a neutral or slightly alkaline Ringer's solution had a *more* pronounced effect on determining prolongation of kidney allograft survival than in an acid Ringer's solution. Flushing the kidney with Con A at a lower pH (5.6) did not prolong survival as well as in the previous groups. So and Goldstein²⁵ have already demonstrated that the binding of Con A to methyl- α -D-mannopyranoside is pH dependent and was maximal at pH 6.2. Our *in vivo* studies indirectly confirmed their observations that very low or high pH reduced the maximal binding properties of Con A.

Finally, our experiments show that it is important to control the manufacturing lot of Con A used in order to achieve consistent results in modifying graft immunogenicity. Recent lots of Con A (1976-77) have produced more variable results than were obtained with the original lots used when these experiments began in 1973. Our recent experience indicates that unless Con A is further purified (Fraction II), it does not prolong graft survival. These results may explain the difficulties that other investigators encountered in demonstrating prolonged survival in the rat model.^{22,23} Further studies in our laboratories are investigating the effect of endotoxin on the Con A samples as the possible reason for the lack of consistency obtained by other researchers.^{22,23}

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