Clinical Effect of Allopurinol on Preserved Kidneys:

A Randomized Double-blind Study

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We investigated the clinical posttransplant effect of allopurinol on preserved kidneys. Thirty-four paired kidneys from brain-dead cadavers were preserved by hypothermic pulsatile perfusion with silica gel fraction in separate cassettes. Allopurinol was added to one perfusate and omitted from the other in a randomized, double-blind, prospective plan. There was no difference in the short-term or long-term function of either group of kidneys. Allopurinol does not appear to have as consistent a beneficial effect on non-ischemically damaged human kidneys as that observed experimentally on ischemically damaged canine organs.

THE INCIDENCE of acute tubular necrosis (ATN) after cadaver kidney preservation varies from 20 to 55%[2,4,5,11] depending on the drug treatment before or during organ retrieval, the type of preservation system, and the length of ischemia and perfusion time. In our laboratory, we noted that allopurinol, a xanthine-oxidase blocker, protected canine kidneys from 60 minutes at 25° warm ischemia damage.[23] Encouraged by our previous findings, we tested the clinical effect of allopurinol during renal preservation, in a randomized prospective double-blind system to determine if allopurinol in the perfusate reduced the incidence of ATN.

Materials and Methods

In a period of 7½ months (Sept. 30, 1974–May 15, 1975) 34 cadaver kidneys from 17 consecutive donors acquired by our donor team were included in a double-blind, randomized study to determine the effect of allopurinol on renal preservation. Kidneys harvested by other teams were not included in this study. Seventeen kidneys were treated with allopurinol during preservation and the other 17 kidneys served as controls and received no allopurinol. All kidneys were harvested and preserved by hypothermic pulsatile perfusion before they were transplanted to patients with end-stage renal failure at our medical center and other medical institutions. All recipients were evaluated for at least 8 months after transplantation. The actuarial survival of all kidneys was calculated by the method of Merrell and Shulman,[10] and functional survival included all losses either due to technical failure, preservation failure, graft rejection, or patient death. Kidney function, tissue matches, kidney loss, warm and cold ischemia time, recipient's age, and acute tubular necrosis were considered in the final comparison, as was length of preservation. The surgeons were not aware which kidney was used for which recipient until the code was broken in December of 1975.

The standard technique of kidney retrieval was used in all brain-dead heart beating cadaver donors. After nephrectomy, the blood was flushed from the organ with cold (4°) lactated Ringer's solution containing 10,000 units/liter of heparin sodium and 0.1 g/L of procaine hydrochloride, until the venous effluent ran clear. Kidneys were preserved in the Mox-100 perfusion system at a systolic perfusion pressure of 60 mm Hg; oxygen pressure (O_2), 200 mm Hg; pH, 7.4 and temperature of 7°. Silica gel fraction (SGF) of plasma[16–18] was used as a

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regular perfusate. SGF contains normal concentrations of extracellular electrolytes and an osmolarity of 285–300 mOsm/liter. Phenolsulfonphthalein, 1 ml/liter; magnesium sulfate 50%, 2 ml/liter; regular insulin, 80 units/liter; ampicillin sodium, 1 gm/liter; and methylprednisolone, 750 mg/liter were routinely added to the perfusate.

Two kidneys from the same cadaver donor were placed in separate preservation casettes. The same additives were used in each kidney except one, which also received 250 mg/L of sodium allopurinol (Burroughs Wellcome Co.). Perfusion pressure, perfusate flow, blood gases, electrolytes, and lactic acid dehydrogenase (LDH) levels were determined every 2 hours for 6 hours, then every 6 to 12 hours in the perfusate until the end of the study. The Mox-100 transport module[12] was used to send kidneys to other transplant centers without interrupting perfusion.

The time that elapsed from the clamping of the renal artery, or circulatory failure in the donor, until the kidney was washed free of blood was recorded as the warm ischemic time. The moment the kidney was flushed clear with iced lactated Ringer's solution until the moment of revascularization in the recipient was recorded as the cold ischemia time.

Histocompatibility testing was performed on peripheral blood donor lymphocytes of lymph node cells removed at the time of nephrectomy. Recipient cytotoxic antibodies directed against donor cells were studied by the microlymphocytotoxicity method of Amos and associates.[1] No patient with cytotoxic antibodies to the donor tissue received a kidney graft.

Operative transplant technique, immunosuppression, and postoperative treatment remained relatively constant.[15] Renal function was defined as that sufficient to sustain life and not require hemodialysis. Permanent reinstitution of dialysis, graft removal, or patient death were all considered graft failure. The diagnosis of ATN is a clinical one, and designates these patients who require at least one dialysis treatment after transplantation and before regaining renal function.

TABLE 1. *Differences Between Control and Allopurinol Treated Kidneys (Average Values)*

	Recipient		Number HLA identities with donor		Preservation Time (hrs)
	Age (*mean* ± SE)	Sex (#)	≤1	≥2	(mean ± SE)
Control (17 kidneys)	32.5 ± 3.3	M (13) F (4)	6	5	24.9 ± 1.2
Allopurinol (17 kidneys)	34.1 ± 2.1	M (14) F (3)	6	4	32.5 ± 4.0

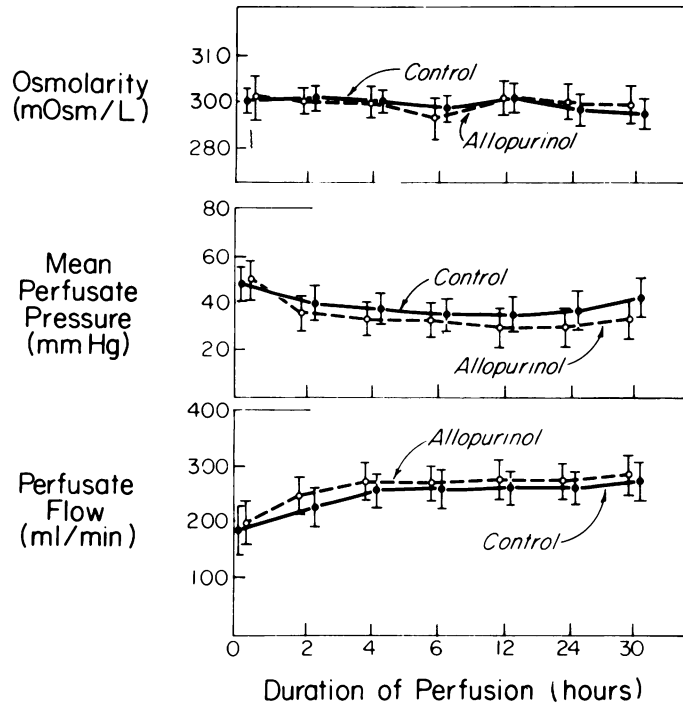

FIG. 1. Osmolarity, perfusate pressure and flow during perfusion of human kidneys with and without allopurinol.

Results

There were no significant differences in age, sex, antigen matching, preservation time and length of ischemia between the control and allopurinol treated kidneys (Table 1). Warm ischemia was less than 5 minutes in all cases. None of the donors was in clinical shock prior to or during donation. There was no warm ischemia or hypotension observed in either group of kidneys. The average preservation time of the allopurinol treated kidneys was 8 hours longer (32 hours) than the other group (24 hours). There were no significant differences in osmolarity, mean perfusate pressure or perfusate flow at any time (Fig. 1). Although slightly modified, the differences between the pH, potassium and LDH also were not significant, even in the allopurinol treated kidneys (Fig. 2).

There were no significant differences in either long-term or short-term function of those kidneys perfused with allopurinol and those untreated kidneys. The time of onset of diuresis, the time at which creatinine clearance exceeded 5 ml/min, the average number of dialyses required were almost identical in both groups. There was no difference in the incidence of ATN in any group even though those kidneys perfused with allopurinol were perfused for longer periods of time (Table 2). Two kidneys perfused with allopurinol and transplanted at other institutions never functioned. Only one kidney perfused without allopurinol and transplanted at another institution never functioned. All kidneys transplanted at the Uni-

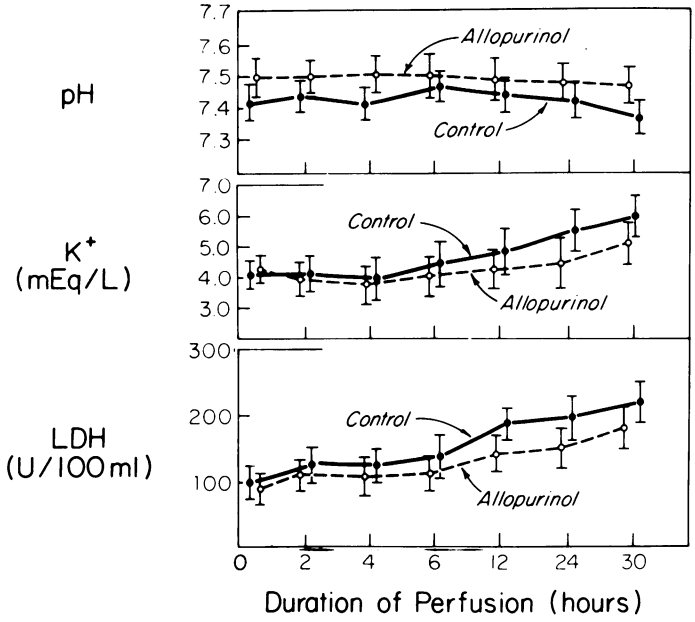

FIG. 2. pH, potassium and LDH during perfusion of human kidneys perfused with and without allopurinol.

versity of Minnesota achieved normal function and only 1 of 15 kidneys has been lost for any reason in the following 8–16 months (Fig. 3).

Discussion

Allopurinol, a xanthine-oxidase inhibitor, blocks the synthesis of uric acid, thereby increasing the accumulation of xanthine, hypoxanthine and other purine nucleotides[7] within the perfused tissue. The exact mechanism by which allopurinol prevents the irreversible damage that occurs after severe ischemic injury is not known. DeWall et al.[6] and Vasko and associates,[25] after studying the response of the ischemic canine myocardium and kidney to allopurinol, suggested that the recovery of the damaged organ was based on the preservation of the pool of functional purine bases that would allow for an increase in the formation of high energy nucleotides. These purine intermediates can be converted to adenosine triphosphate (ATP), if sufficient oxygen becomes available after the initial ischemic injury. We have demonstrated that canine kidney damage produced by 60 minutes of warm ischemia at 25° could be prevented if, before nephrectomy, the donor dog was first treated with allopurinol, and the excised kidneys were then perfused with a plasma perfusate combined with allopurinol.[20,23]

Experimentally, the protective effect of allopurinol on ischemic kidneys[3,14,20,23,25] ischemic hearts,[6] small bowel,[22] and liver[24] has been demonstrated.

The severity of damage increases with the degree and duration of ischemia. Murdock and Cho[13] demonstrated that perfused dog kidneys did not respond to allopurinol

TABLE 2. *Proportion of Recipients of Preserved Kidneys Requiring At Least One Dialysis*

Perfusate	Perfusion Length ≤24 hr	>24–30 hr	>30–40 hr	>40–70 hr
With Allopurinol (17)	2/6	1/2	2/4	4/5
Without Allopurinol (17)	4/6	2/9	2/2	0/0

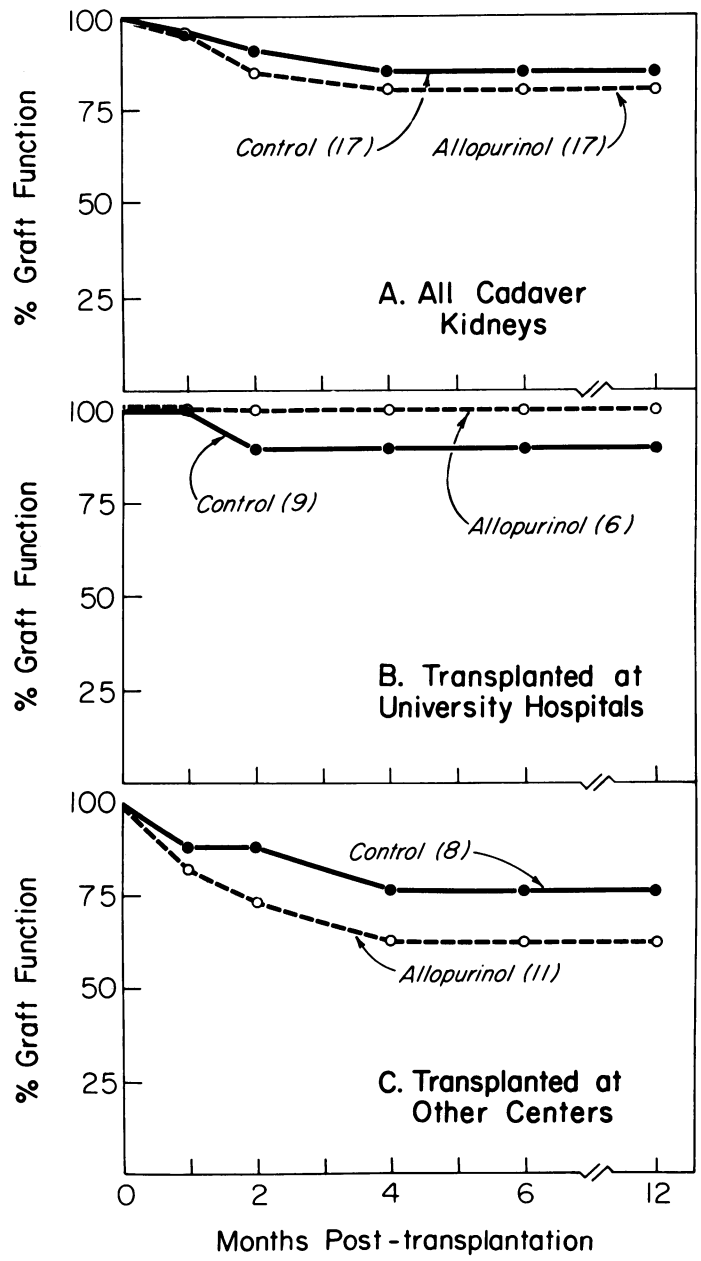

FIG. 3. Long-term function of perfused cadaver kidneys. All kidneys have been followed at least 7½ months. All causes of kidney loss including patient death are recorded.

when the ischemia was increased to 37° for one hour. An ischemic injury of such a magnitude (37°) for one hour, followed by 24 hours of hypothermic pulsatile perfusion, caused irreversible damage.[21]

Toledo-Pereyra and colleagues[19] did not observe any reconversion of purine intermediates to ATP during the hypothermic perfusion of ischemic kidneys treated with allopurinol. Immediately after revascularization, however, the ATP levels were significantly higher in the allopurinol treated kidneys than in the untreated group.[19] Keaveny, Cunningham and Fitzgerald[8] also obtained higher levels of ATP after treatment of ischemic rat kidneys with allopurinol. Lindsay and associates[9] recently demonstrated higher concentrations of ATP, and lesser levels of potassium and lactic acid on ischemic canine hearts chronically treated with allopurinol.

We could not confirm our animal studies in this prospective randomized trial in human cadaver kidneys. Several factors of this clinical study differ from our experimental protocol. First of all, there was no period of warm ischemia to the cadaver kidneys that would be expected to result in renal damage such as that deliberately inflicted on the animal kidneys for experimental purposes. Secondly, the human cadaver donors were not pretreated with allopurinol and the amount of allopurinol used during preservation was half the dosage we used in the laboratory model in order to assure the absence of damage to the kidney due to the allopurinol. Thirdly, the plasma perfusate (SGF) in this clinical study varied in some basic characteristics from the cryoprecipitated plasma or human plasmanate used experimentally since we have found that silica gel fraction plasma perfusate is superior to standardized cryoprecipitated plasma for preservation. Finally, the length of preservation was longer in the human kidneys treated with allopurinol (32 hours, average) than in the group of canine kidneys (24 hours).[20,23] These factors probably account for some of the differences observed in the clinical study. We can say that allopurinol in this dose did not apparently damage the kidneys and we cannot determine whether it will be of value in those circumstances in which warm ischemia damage occurs.

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