Effect of Allopurinol on the Preservation of Ischemic Kidneys Perfused with Plasma or Plasma Substitutes

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Canine kidneys were autotransplanted into nephrectomized dogs after exposure to room temperature for 40–90 minutes and preservation for 24 hours by hypothermic bloodless pulsatile perfusiin. Three perfusates were used; cryoprecipitated pooled dogs' plasma, human albumin, and human plasmanate. Perfusion after periods of warm ischemia resulted in irreversible renal damage, unless allopurinol was added to the perfusate and fed to the dogs. Thus, metabolic manipulation is capable of protecting tissues in the face of severe ischemic insult, even when using plasma substitutes for perfusion.

KIDNEY PRESERVATION for periods of 24–48 hours is now routine clinical practice,[2] the most important limiting factor being the period of warm ischemia prior to preservation.[3] We have previously demonstrated that the treatment of canine kidneys with allopurinol during perfusion with cryoprecipitated plasma significantly minimized the damage induced by a one-hour period of warm ischemia.[10] Furthermore, if the animal was given allopurinol after transplantation, in addition to the perfusion treatment, there were no deaths related to severe renal ischemia.[10]

A second problem remains in preservation; the development of plasma substitutes to eliminate the time-consuming and costly method of plasma cryoprecipitation. Plasma substitutes can be used, but not if a prolonged period of warm ischemia precedes preservation.[7] The present study was designed to assess the effect of allopurinol in protecting against ischemic damage when added to a perfusate of plasma or plasma substitutes.

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Materials and Methods

Thirty-six adult mongrel dogs weighing 15–24 kg were anesthetized with sodium methohexital for induction and with halothane for maintenance. Through a left lateral subcostal incision, the left kidney was removed and placed at 25 C for a period of time ranging from 40 to 90 minutes. It was then flushed with cold (4 C) Ringer's lactate solution containing heparin (10,000 U/L) and procaine (1 gm/L) until the venous effluent was clear. Subsequently, each kidney was preserved for 24 hours by pulsatile perfusion at 7 C, pH 7.4, pO_2 200 mm Hg, pulse rate 60 and systolic perfusion pressure of 60 mmHg.[9] The perfusion system was primed with 700 ml of the perfusate to be used. Flow rate, perfusion pressure, kidney weight and perfusate electrolytes and lactic acid dehydrogenase (LDH) were determined every six hours. After preservation and autotransplantation, immediate contralateral nephrectomy was performed.

Six groups of kidneys were included with six kidneys in each group. Groups I and II were exposed to 90 minutes of warm ischemia and perfused with cryoprecipitated plasma prepared by Belzer's technique.[1] Groups III and IV were exposed to 60 minutes warm ischemia and were perfused with human albumin 25% (Cutter Laboratories). Groups V and VI were exposed to 40 minutes warm ischemia and perfused commercial human plasmanate (Cutter Laboratories). Methylprednisolone (500 mg/L) was added to all perfusates. Allopurinol (500 mg/L) was added to the perfusate of kidneys in Groups II, IV and VI. The animals in Groups II, IV and VI were fed allopurinol (100 mg/kg/day) for four days prior to and 10 days after transplantation.

TABLE 1. *24 Hour Perfusion Characteristics of the Control and the Kidneys Treated with Allopurinol* (Mean Values ± SD)

Groups	Perfusates	Allopurinol Treatment	Flow Rate (ml/min/gm) Initial Value	Flow Rate (ml/min/gm) Final Value	K+§ (mEq/L) Initial Value	K+§ (mEq/L) Final Value	LDH‖ (U/100ml) Initial Value	LDH‖ (U/100ml) Final Value	Weight Gain (% from Control) Final Value
I	CPP*	−	0.7 ± 0.2	0.6 ± 0.3	4.5 ± 0.7	6.2 ± 1.0	82 ± 35.1	175 ± 17.5	8.5 ± 1.5
II	CPP*	+	0.7 ± 0.2	1.2 ± 0.2	5.3 ± 0.6	5.9 ± 1.4	65 ± 28.4	200 ± 19.5	7.2 ± 2.0
III	A†	−	0.9 ± 0.2	0.8 ± 0.3	5.5 ± 1.2	6.4 ± 1.2	75 ± 15.7	185 ± 25.5	10.5 ± 1.2
IV	A†	+	1.0 ± 0.2	1.4 ± 0.3	4.2 ± 1.3	5.7 ± 1.2	56 ± 25.7	275 ± 12.5	8.5 ± 2.3
V	P‡	−	0.4 ± 0.1	0.5 ± 0.2	4.5 ± 0.7	6.2 ± 0.9	85 ± 30.5	135 ± 15.8	11.5 ± 3.0
VI	P‡	+	0.5 ± 0.2	0.9 ± 0.3	4.3 ± 0.7	6.7 ± 1.1	74 ± 21.3	145 ± 18.5	9.8 ± 2.5

* Cryoprecipiated plasma (90 min. ischemia).
† Human Albumin (60 min. ischemia).
‡ Human plasmanate (40 min. ischemia).
§ Normal values 3.8–5.2 mEq/L.
‖ Normal values 40–80 U/100 ml.

All animals were examined daily until death. Renal function was determined by daily serum creatinine (normal 0.6 to 1.6 mg%) and uric acid (normal 3.2 to 7.5 mg%) levels. The experiment was terminated 30 days following transplantation. Postmortem examination with kidney biopsy was performed in all cases.

Results

The perfusion characteristics of the kidney in each group are shown in Table 1. All kidneys demonstrated poor initial post-ischemic perfusate flow which remained the same or even deteriorated in kidneys not perfused with allopurinol. When allopurinol was added to the perfusates, a significant improvement in perfusate flow was noticed by 24 hours of perfusion. The other perfusion parameters (weight gain, potassium and LHD), however, did not improve with the addition of allopurinol (Table 1). In addition, there were some differences in perfusion characteristics depending on the type of perfusate used; for example, the kidneys with the lowest flow and greatest weight gain were perfused with plasmanate. All kidneys demonstrated high perfusate potassium and LDH levels by the end of the 24 hours perfusion.

Table 2 summarizes the state of renal function and survival of dogs autotransplanted with preserved kidneys after periods of warm ischemia. Dogs autotransplanted with kidneys perfused with albumin or plasmanate were almost all dead of uremia by 30 days. Eighty-four per cent of cohort dogs survived if allopurinol had been added to the perfusate. Similarly, only one of six kidneys perfused with cryoprecipitated plasma after 90 minutes warm ischemia was capable of supporting life. All kidneys perfused with cryoprecipitated plasma plus allopurinol functioned normally by two weeks after transplantation.

Discussion

It is well known that hypoxic tissues lose purine intermediate compounds to the surrounding fluids, but recover and reconvert them if oxygen is restored,[4,6] since the cell possesses resynthesis pathways such that hypoxanthine and higher compounds can be reconverted to ATP.[12] Once degradation of the nucleotides has proceeded beyond the xanthine level, however, they become irreversibly lost to the nucleotide pool.[5,11] Allopurinol, a xanthine oxidase inhibitor, appears to act by inhibition of the further degradation of xanthine. DeWall and associates[5]

TABLE 2. *Survival and Renal Function of Kidneys Autotransplanted After Warm Ischemia and 24 Hypothermic Pulsatile Perfusion*

Group	Perfusates	Ischemia Time (min)	Allopurinol Treatment	Serum Creatinine at one week§	Serum Uric Acid at one week§	Days to return to Normal Serum Creatinine‖	Survival**
I	CPP*	90	−	8.2 ± 1.5	5.6 ± 1.0	No return	16% (1/6)
II	CPP*	90	+	2.1 ± 0.9	4.3 ± 0.7	9.5 ± 1.8	100% (6/6)
III	A†	60	−	12.1 ± 2.5	4.8 ± 1.6	No return	0% (0/6)
IV	A†	60	+	2.9 ± 0.8	3.9 ± 0.3	13.1 ± 8.7	84% (5/6)
V	P‡	40	−	11.8 ± 1.7	5.6 ± 1.0	No return in 5.	0% (0/6)
VI	P‡	40	+	1.8 ± 0.5	4.1 ± 0.7	15 days in one. 9.5 ± 1.8	84% (5/6)

* Cryoprecipitated plasma
† Human Albumin
‡ Human Plasmanate
§ mg% (Mean values ± SD)
‖ Mean values ± SD
** More than 30 days with normal creatinine

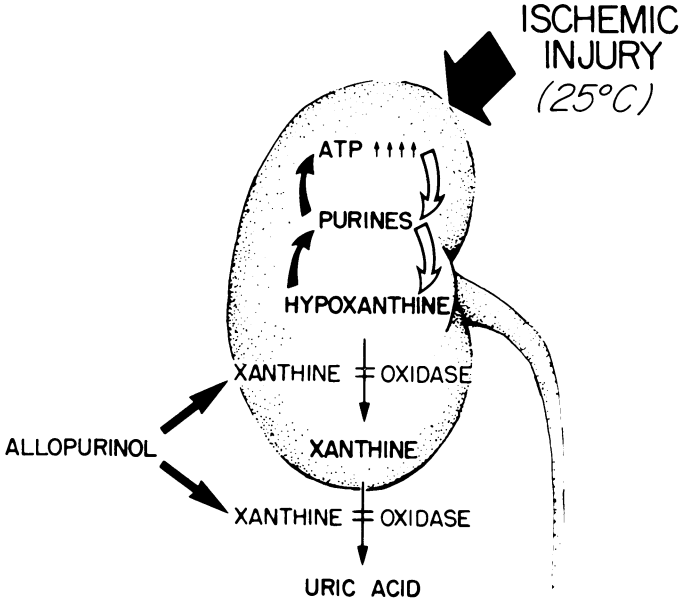

FIG. 1. General metabolic pathway of the purines when degradation has been inhibited by allopurinol during hypoxia. Allopurinol appears to prevent ATP loss in the ischemic kidney.

have studied the response of the ischemic myocardium to allopurinol. They stated that the recovery of the damaged muscle is based on the utilization of the pool of functional purine bases which would be available for reformation of high energy nucleotides. A similar sequence probably takes place in the ischemically damaged kidney. The treatment of the kidneys with allopurinol probably allows the purine intermediates to be reconverted to ATP, if sufficient oxygen becomes available after the initial ischemic injury (Fig. 1).

Warm ischemic damage of the preserved kidneys is an important cause of injury, the severity of which increases with the duration of ischemia. The absence of oxygen precludes the utilization of the citric acid cycle and increases anaerobic metabolism with accumulation of lactic acid and other anaerobic by-products. The maintenance of celluar volume is an active energy consuming process which is dependent on the sodium pump.[8] Lack of energy causes pump failure which leads to accumulation of sodium and water in the cells and cellular swelling. Continuous hypothermic perfusion with various oxygenated perfusates permits only minimal cellular swelling and partially reverses the anoxic injury by allowing aerobic metabolism to recommence. Although certain perfusates are better than others, a kidney subjected to room temperature for more than 60 minutes without oxygen fails to sustain life if the kidney is then perfused for 24 hours with either cryoprecipitated plasma or albumin. Even periods of ischemia as short as 40 minutes seriously damage kidneys perfused with plasmanate.

This study confirms the protective effect of allopurinol in the prolongation of tissue and organ survival time in the face of hypoxia even when less than optimal perfusates are utilized. The potential immediate clinical application to transplantation is obvious.

In addition, allopurinol may well prove to be a useful additive in the search for simple, less expensive and readily available substrates for cryoprecipitated plasma as a perfusate for organ preservation.

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