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Failure of Inosine to Prevent Ischemic Damage in the Canine and Rat Kidney Models

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Summary. Intravenous, renal arterial, and intraperitoneal administration of inosine at dosages of 100 to 200 mg/kg, 160 mg/kg, and 200 mg/kg, respectively, in the dog and rat was used to test its efficacy in preventing ischemic damage after 60 min of warm ischemia in an in situ solitary renal model. No improvement of renal function as compared with a control and a conventional mannitol/furosemide treatment group was detected. Rather, inosine at dosages of $\geqslant$160 mg/kg resulted in significantly impaired renal function in the experimental groups of the dog model; no improvement was observed in the rat model. These results suggest that the use of inosine in human renal surgery and preservation should be approached cautiously.

Key words: Inosine, Renal ischemia, Renal ischemic damage, Renal transplantation.

Introduction

The majority of kidneys for transplantation come from cadaveric donors and warm ischemia continues to limit the time for safe preservation. Ischemia associated with harvesting and its deleterious effects on renal viability continue to be a problem. In this regard, several studies have evaluated the effect of inosine in the prevention of ischemic damage, with or without cold storage preservation, and results have been conflicting [1, 2, 4, 5, 15]. Several investigators have shown experimentally that kidneys pre-treated with inosine have superior post-ischemic renal function when compared with untreated controls [1, 4, 11], but such pre-treatment was not useful for kidney preservation [6, 8–10, 12]. This controlled study was undertaken to evaluate in canine and rat kidney models whether, in fact, the use of inosine at various dosages offers any protection equal to or better than that achieved with conventional mannitol-furosemide.

Material and Methods

Dogs. Seventeen adult, female, well-conditioned, mongrel dogs (14 to 18 kg) were utilized. Anesthesia was initiated with Nembutal and maintained with Fluothane 0.3%, delivered continuously by a Bird respirator via an endotracheal tube. Throughout the procedure, 0.9% saline (100 ml/kg) was used to maintain intravascular volume. A transabdominal midline incision was employed. The renal artery and vein and the ureter of the isolated left kidney were occluded to induce ischemia in all animals. After 60 minutes, the clamps were removed, and immediate contralateral nephrectomy was performed. Serum creatinine and blood urea nitrogen were measured immediately preoperatively and on days 1, 2, 3, and 7. Five separate experiments were performed in dogs, and all animals received heparin, 400 units/kg intravenously, 20 min before ischemia, as follows:

Group 1 (control, $N = 4$): The only treatment was 500 ml of 0.9% saline given intravenously before ischemia.

Group 2 ($N = 2$): The combination of 12.5 g of mannitol and 20 mg of furosemide in 500 ml of 0.9% saline was given intravenously 15 min before ischemia.

Group 3 ($N = 6$): Inosine, 100 mg/kg in 500 ml of 0.9% saline, was infused intravenously 20 min before ischemia.

Group 4 ($N = 2$): Inosine, 200 mg/kg in 500 ml of 0.9% saline, was infused intravenously 20 min before ischemia.

Group 5 ($N = 3$): Inosine, 160 mg/kg (as a 4% solution in 0.9% saline), was injected into the left renal artery before ischemia with a no. 21 butterfly needle; up to a volume of 500 ml of 0.9% saline was given intravenously.

Rats. Experiments were performed in 17 female Sprague-Dawley rats with the use of ether for anesthesia. Two experiments were performed as follows:

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Table 1. Serum creatinine and blood urea nitrogen values in dogs undergoing renal ischemia in situ (mean)

Treatment group	No.	Serum creatinine, mg/dl			Blood urea nitrogen, mg/dl			Survival (%)
		Preop	Max.	Final	Preop	Max.	Final	
Group 1 (control)	2	0.98	1.70	1.22	15.15	36.20	19.00	100
Group 2 (mannitol and furosemide)	4	0.72	1.34	1.20	13.75	29.40	25.82	100
Group 3 (inosine, 100 mg/kg intravenously)	6	1.02	1.68	1.45	17.48	31.02	20.92	100
Group 4 (inosine, 200 mg/kg intravenously)	2	0.82	3.22	1.75	26.50	67.75	39.05	100
Group 5 (inosine, 160 mg/kg intra-arterially)	3	0.83	3.15	2.30	16.00	63.83	46.83	100

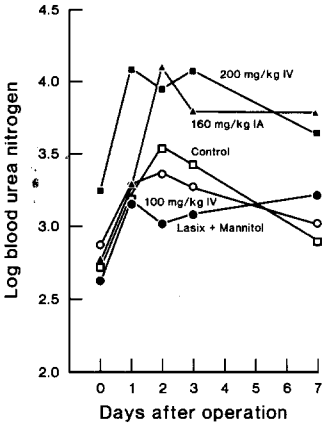

Fig. 1. Effect of inosine on blood urea nitrogen concentration in dogs as a function of time after renal ischemia. Control, only 0.9% saline administered; Lasix + mannitol, 20 mg of furosemide and 12.5 g of mannitol in 500 ml of 0.9% saline, given intravenously 15 min before ischemia, *IV*, intravenous; *IA*, intra-arterial

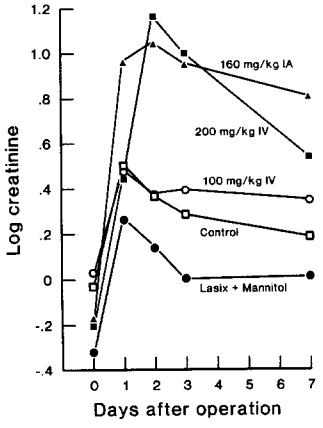

Fig. 2. Effect of inosine on serum creatinine concentration in dogs. See legend to Fig. 1 for details

Group 1 (control, $N = 9$): After atraumatic isolation of the left kidney, renal vessels, and ureter, 3 ml of 0.9% saline was administered intravenously (right renal vein) 40 min before the left renal vessels and ureter were clamped for 60 min in situ. Contralateral nephrectomy was performed.

Group 2 ($N = 11$): These rats received inosine, 100 mg/kg body weight, as a 1% solution in saline intraperitoneally 40 min before the left renal pedicle and ureter were clamped for 60 min in situ and contralateral nephrectomy.

All animals were observed for 10 days postoperatively, and renal function was tested on days 1, 3, and 10. All received heparin 150 units intravenously before clamping of the renal pedicle and ureter.

Analysis of the data on serum creatinine and blood urea nitrogen in dogs was based on a log transformation of the observed values (days 0, 1, 2, 3, and 7). Multivariate analysis of variance was used to compare the groups of dogs with respect to the mean, linear, and quadratic aspects of their observed profiles. For the rat data (log-transformed), values were compared on day 3 and on the basis of the change from day 3 to day 10 with the use of an independent two-sample t test.

Results

Dogs. The mean preoperative, final, and maximum serum creatinine levels and blood urea nitrogen values in their original scale are listed in Table 1. No dog died during the period of observation. The observed mean profiles for the log-transformed data are shown in Figs. 1 and 2. The (overall) multivariate F test indicated differences among the groups ($P < 0.001$) when the three polynomial aspects (mean, linear, and quadratic) of serum creatinine and blood urea nitrogen were considered simultaneously. Duncan's multiple-range test [14] (at $\alpha = 0.05$), applied to the polynomial aspects of each response type separately, indicated that groups 1, 2, and 3 were collectively different from groups 4 and 5 with respect to all three aspects of the serum creatinine profiles. For blood urea nitrogen, groups 4 and 5 were different from group 1 with respect to the mean and quadratic aspects, but groups 4 and 5 were not significantly different from groups 2 and 3.

Rats. The data on serum creatinine and blood urea nitrogen were analyzed and compared on postoperative day 3 for the two groups of rats. The groups were also compared on the basis of the changes from day 3 to day 10 for these variables. The results are listed in Table 2 for the mean (±SE) of the log-transformed data for both serum creatinine and blood urea nitrogen. Inosine did not appear to have a protective effect on the ischemically injured rat kidneys.

Table 2. Serum creatinine and blood urea nitrogen (log-transformed) values in rats undergoing renal ischemia in situ (mean ± SE)[a]

Treatment group	No.	Day 3		Day 10 – day 3	
		Creatinine	Blood urea nitrogen	Creatinine	Blood urea nitrogen
Control (saline intravenously)	9	0.57 (± 0.30)	4.26 (± 0.32)	−0.56 (± 0.19)	−0.42 (± 0.19)
Inosine (200 mg/kg intraperitoneally)	11	0.49 (± 0.25)	4.28 (± 0.28)	−0.83 (± 0.25)	−0.85 (± 0.27)

[a] Differences between groups in each category were not statistically significant

Discussion

Continuous hypothermic pulsatile perfusion and simple flushing of the kidney with a chilled intracellular solution followed by cold storage is still the major method of preserving cadaver kidneys before transplantation. Presently, approximately 15% of cadaver kidneys never function after transplantation [3]. In an effort to improve functional graft survival in recipients of cadaver kidneys, inosine, a purine nucleotide, has been used in clinical renal surgery as an agent with which to enhance ischemically injured kidneys [13], although its usefulness is controversial on the basis of studies in several animal models [1, 2, 4, 5, 15].

The advocates of inosine reported superior post-ischemia renal function in comparison with untreated controls [1, 4, 11]. Fernando et al. [4] reported that inosine enhanced renal function after 60 min of normothermic ischemia in both dogs and rats. Rothwell et al. [11] reported that direct intrarenal perfusion of inosine was protective for the canine kidney after 90 min of warm ischemia. They added that the serum creatinine levels were significantly lower postoperatively in an inosine-treated group as compared with a control group, and they were able to confirm this result on histologic examination.

However, other investigators have failed to note a beneficial effect of inosine in kidney preservation [6, 8, 9, 12]. Also, Buhl and associates [1] demonstrated that pretreatment intravenously with inosine (100 mg/kg) before hypothermic flushing and then simple cold storage for 22 h in an intracellular solution with 0.5 to 2.5 mM inosine had no beneficial effect on renal preservation. Marshall and coworkers [10] demonstrated a decline in renal function to near zero after 90 min of warm ischemia in isolated perfused rat kidneys, noting that total sodium reabsorption was the most sensitive indicator of renal damage. They concluded that inosine had no effect on subsequent renal function after 60 or 90 min of warm ischemia or after 24 h of cold storage ischemia.

Various pharmacologic agents have been used in an attempt to ameliorate the ischemically damaged kidneys during preservation. Results have been equally variable. Green and associates [7] investigated the effects of diuretics, vasoactive agents, and membrane stabilizers in their ability to ameliorate severe ischemia injury (1 h at 37 °C) in the rabbit model. According to their findings, only the diuretics mannitol and furosemide produced a significant improvement in renal function when they were given before the ischemic injury. Casali et al. [2] did not find that inosine improved renal function in a dog model but it did do so in the monkey model. They concluded that the protective effects of inosine on renal warm ischemia may be dependent on the species, the route of administration, and the dosage of the drug.

In our present study, at dosages described before [4, 5], inosine did not enhance renal function in pretreated dog or rat kidneys subjected to 60 min of in situ normothermic ischemia. Rather, at dosages $\geqslant$ 160 mg/kg, its effect on renal function was a deleterious one, and it significantly impaired renal function as compared with controls and animals treated with mannitol/furosemide. Its use in the preservation of human cadaver kidneys and in clinical surgery should thus be approached cautiously.

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