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Preservation of the Ischaemic Canine Kidney with Inosine

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Summary. Direct intra-renal perfusion of inosine was found to protect the canine kidney from 90 min of warm ischaemia.

Significantly lower ($P < 0.01$) serum creatinine levels were found 24 and 72 h post-operatively in an inosine treated group (N = 12) when compared to a control group (N = 5).

Histological examination of the pre-treated kidneys confirmed the protective effect of inosine.

Key words: Inosine, Canine kidney, Preservation.

INTRODUCTION

Previous workers (1, 3, 4, 7) have shown both experimentally and clinically that kidneys pre-treated with the purine nucleoside, inosine, have a superior post-ischaemic renal function when compared to untreated controls.

Inosine is thought to promote rapid regeneration of nucleotides (1, 4) thereby improving cell viability in the post-ischaemic kidney.

The clinical application of this concept has been explored in renal staghorn surgery (7) and found to be successful after 60 min of warm ischaemia. However, situations may arise in this type of surgery where the kidney is ischaemic for up to 90 min and permanent damage may be the result.

This study was undertaken to define the structural and functional changes of the canine kidney after 90 min of warm ischaemia, and to determine the efficacy of inosine pre-treatment in preventing these changes.

MATERIALS AND METHODS

Seventeen male adult dogs of 20-25 kg were divided into 2 groups (Table 1). Twelve hours before surgery food and water were withheld from the animals. Anaesthesia was induced by thiopentone (22 mg/kg) and maintained with a mixture of nitrous oxide, halothane and oxygen. Both kidneys were exposed through a 15 cm incision along the linea alba. The left renal artery was freed from surrounding adventitial tissue, occluded with a bulldog vascular clamp placed 0.5 cm distal to the aorta, and the kidney perfused with either a 4% solution of inosine (12 dogs), or 60 mls of 0.9% NaCl (5 dogs), through a 23 gauge butterfly needle. The inosine solution was prepared in a 5% dextrose solution at 37°C and administered in a dose of 160 mg/kg (4). After perfusion the left renal vein was also occluded with a bulldog clamp, and the contralateral kidney was then excised.

Ninety min later the clamps on the left renal vessels were removed and 4 ml of ampicillin (125 mg/ml) were administered parenterally. Five ml blood samples were collected pre-operatively and on days 1, 4 and 7 after surgery. The levels of creatinine in these samples were determined using a multiple sequential analyser (Technicon 12/60).

On day 7 or earlier if an animal was *in extremis*, the dogs were killed with 1 gm of pentobarbitone sodium (Euthesate, Vetco-Products, Auckland, New Zealand) injected intravenously. The left kidney was examined grossly and a 0.5 cm thick tissue slice of the cortex and medulla was fixed in Helly's fluid (2) for 3 h, washed in water for 24 h and processed for histology. 2 µm and 4 µm thick sections of paraffin embedded tissue were stained with haematoxylin and eosin, periodic acid-Schiff reagent and azan using standard techniques (2).

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Table 1. Experimental groups

	Group[a]	Unilateral nephrectomy	Renal perfusate	
			Saline	Inosine
Control	(5)	√	√	-
Inosine	(12)	√	-	√

[a]Number of dogs in each group in parenthesis

RESULTS

Four of the five dogs with saline perfused kidneys were killed in extremis between day 3 and day 6 of the post-surgical period. The fifth dog in this group showed a moderate elevation of serum creatinine on the first post-operative day (0. 1 to 0 4 mmol/l), but this level then declined and by day 7 was 0 25 mmol/l. However, the remaining 4 dogs in this group showed a rapid increase in their levels of serum creatinine (Fig. 1), which ranged from 1. 14 to 2. 4 mmol/l (mean 1. 84) when the animals were killed.

In contrast to the above group, only 2 of the 12 dogs with inosine-perfused kidneys failed to survive the experiment and apart from these 2 dogs, all remained clinically normal throughout the post-operative period. These 2 dogs were killed on the fourth post-operative day after showing respiratory difficulties and central nervous system disturbances. The levels of serum creatinine in these dogs were 1. 13 and 0. 93 mmol/l. The remaining dogs in this group showed only a moderate increase in serum creatinine levels on day 1 and day 4 (Fig. 1) and by day 7 these levels had declined. As a group the inosine treated animals, on day 1 and day 4, showed significantly lower ($p < 0.01$, Mann-Whitney U) serum creatinine levels compared to the saline control animals. Too few control animals survived to day 7 for statistical comparison.

The histological appearances of kidneys from saline control and inosine treated dogs that were killed during the experiment were similar. All showed diffuse acute tubular necrosis and congestion of glomerular tufts (Fig. 2). The kidney in the remaining saline control dog that survived the experiment showed mild interstitial oedema and occasional small aggregates of mononuclear cells in the cortical interstitium. However, the glomeruli and tubular epithelium showed a normal appearance. Eight of the 10 inosine-treated kidneys, examined on day 7, showed well preserved glomeruli and tubulus (Fig. 3) apart from a few tubular epithelial cells (10-15% that appeared swollen and vacuolated. A few tubular lumina also contained epithelial cells that appeared to have sloughed off the tubular basement membrane. The cortical interstitium was mildly oedematous and all contained occasional foci of inflammatory cells, which were predominantly of mononuclear type. One kidney also showed increased fibrous tissue in the cortical interstitium. The remaining 2 kidneys in this group showed widespread tubular necrosis and extensive calcification in the outer third of the cortex (Fig. 4) but the inner 2 thirds was of normal appearance.

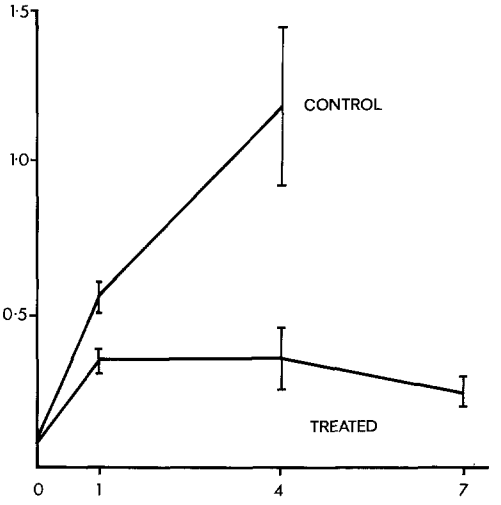

Fig. 1. A graph showing changes in the group means for serum creatinine (mmol/l) in control and inosine treated dogs during the post-operative period. The vertical bars represent one standard error of these means

DISCUSSION

We have demonstrated in this study that inosine perfusion of canine kidneys prior to 90 min of warm ischaemia significantly improved the preservation of their post-ischaemic structure and function. Thus our findings support those of other inosine preservation studies in rats (3) and dogs (4), but in addition we have shown that the period of warm ischaemia may be extended for at least 30 min longer than the 60 min used in the above experiments. Also our results are at variance with those of Woo et al. (8), who failed to observe any beneficial effect in dogs from systemic infusion of inosine prior to 120 min of renal ischaemia.

Nevertheless, despite the significant improvement in post-ischaemic renal function observed in our inosine treated kidneys, all showed areas of tubular necrosis, which in 2 involved most of the outer third of the cortex. These findings indicate the importance of histological assessment of renal injury in preservation studies. Why areas of tubular necrosis should occur in otherwise well preserved tissue remains uncertain, although there was no histological evidence that

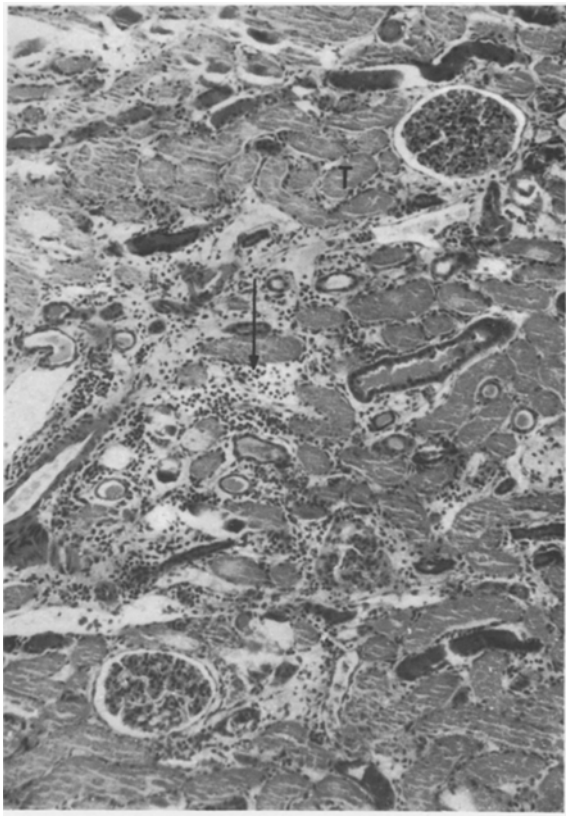

Fig. 2. Renal cortex of a control kidney 4 days after operation showing diffuse necrosis of tubules (T) and an inflammatory cell infiltrate (arrow). (H&E x 120)

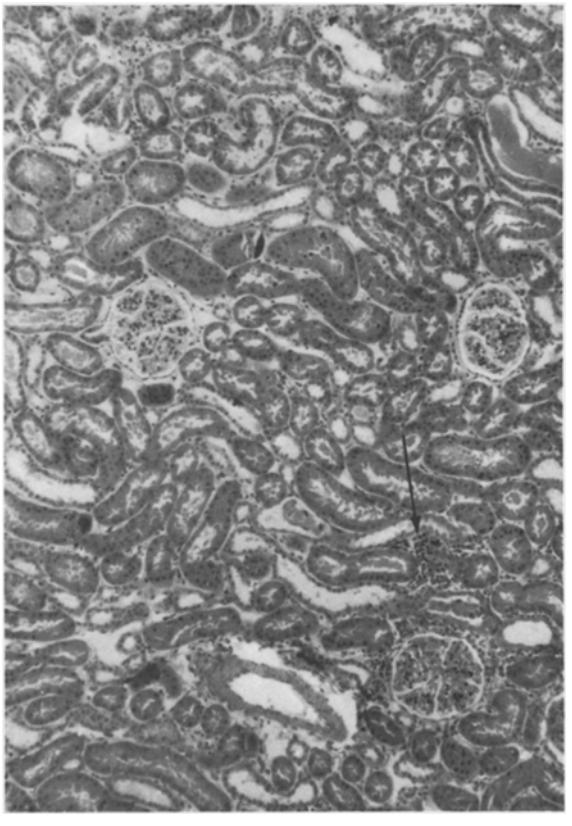

Fig. 3. Renal cortex of an inosine treated kidney 7 days after operation showing glomeruli and tubules of normal appearance and a periglomerular inflammatory infiltrate (arrow). (H&E x 120)

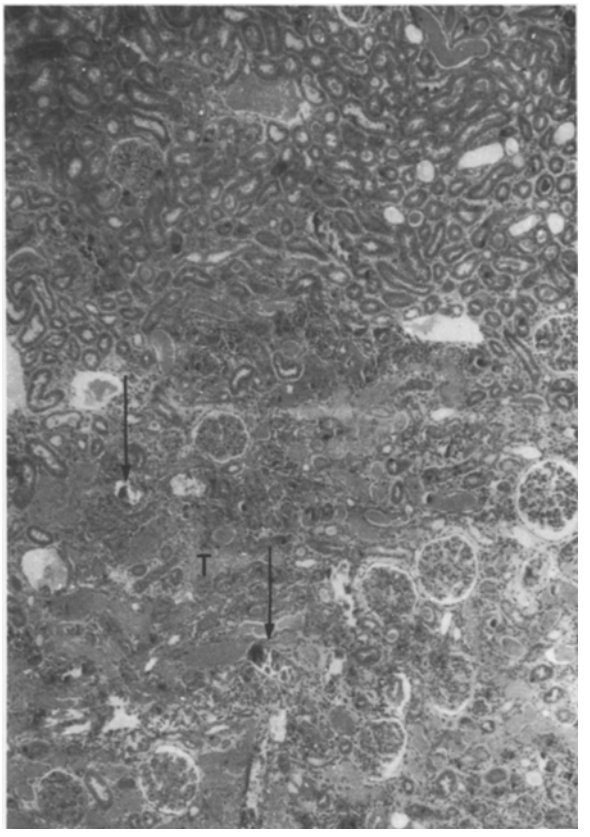

Fig. 4. The outer third of renal cortex of an inosine treated kidney 7 days after operation showing an area of tubular necrosis (T) and calcium within tubular lumina (arrows). (H&E x 100)

the inosine infusion caused vascular thrombosis. However, other possibilities such as uneven distribution of the inosine perfusate within the cortex or patchy cortical blood flow during the post-ischaemic period cannot be excluded in present investigations. Further studies with fluorescent tracers either in the inosine perfusate or in a post-ischaemic blood infusion would help to distinguish between these 2 possibilities.

The mode of action of inosine tissue preservation is speculative at present, but it is likely that several factors may contribute to its beneficial effect. The inosine perfusate used in our studies and those of Fernando et al. (3, 4) was strongly hypertonic (426 mosmol in our study), which may have reduced tubular cell swelling and improved post-ischaemic cortical blood flow (6). This may explain also the improved preserva-

tion that Woo et al. (8) observed in canine kidneys, systemically perfused with a mixture of inosine and mannitol. Nevertheless, inosine administered intraperitoneally appears to be as effective as an intra-renal perfusate (3), and thus hypertonicity of the perfusate is unlikely to be the principle mode of its action. Alternatively inosine may act as an exogenous pool of purine nucleosides which are readily available for resynthesis of adenosine nucleotides, thus by-passing the more demanding adenosine triphosphate (ATP) de novo synthesis pathway, when the blood flow is restored (3). However, preservation with adenosine appears to be less effective than inosine (3), and in our experience (unpublished observations) allopurinol, which should maintain adequate levels of hypoxanthine by blocking xanthine oxidase, provides no appreciable protection. These observations argue against inosine acting simply as a passive provider of preformed purine nucleosides.

There is evidence from experimental studies of myocardial ischaemia in the dog (5) that inosine stimulates the activity of lactate dehydrogenase, succinate dehydrogenase and glucose-6-phosphate dehydrogenase. The activity of this last enzyme is related to the activity of the pentose-phosphate shunt which is of particular importance in the synthesis of nucleic acids and proteins. Thus inosine may have an active role during ischaemia in the activation of enzyme systems and the maintenance of essential cellular functions.

Which, if any, of these postulated mechanisms is important in the ability of inosine to preserve ischaemic tissue remains to be determined. However, the accumulating experimental and clinical evidence on the efficacy of inosine preservation should stimulate more detailed biochemical studies of its mode of action in acute renal ischaemia.

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