DISSOCIATION OF STRUCTURE AND FUNCTION AFTER ISCHAEMIA-REPERFUSION INJURY IN THE ISOLATED PERFUSED RAT KIDNEYS

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Abstract - Oxygen-derived free radicals (OFR) involvement in ischaemia-reperfusion (IR) injury was investigated in a rat isolated kidney model, using 20 minutes ischaemia followed by 15 or 60 minutes reperfusion. Two antioxidants, the xanthine oxidase inhibitor allopurinol and the hydroxyl radical scavenger dimethylthiourea (DMTU), were used to try and prevent OFR-related damage. Renal function was estimated from the inulin clearance, fractional sodium excretion and renal vascular resistance. Location and extent of tubular damage, and type of cell death (apoptosis vs necrosis) were used as morphological parameters of IR-induced change. Cell damage was most extensive in the nephron segments of the outer zone of the outer medulla (straight proximal tubule and thick ascending limb (TAL)). Pre-treatment with allopurinol or DMTU did not improve renal function. Less structural damage was observed in the TAL of allopuriol - or DMTU - treated kidneys compared with IR alone. In allopurinol - treated kidneys, luminal debris was less extensive than that seen in IR kidneys. Most cell death was necrotic in type and morphological features of apoptosis were seen infrequently. The beneficial effects of allopurinol and DMTU on structural change did not correlate with functional improvement during the reperfusion period. This may require longer reperfusion or multiple treatments. The results suggest that OFR - injury is of limited significance in this model of renal IR injury. Targeting OFR injury may only be useful after very brief periods of ischaemia where necrosis is minimal and the potential for recovery is greater. The results confirm the different susceptibilites of individual nephron segments to injury within the intact kidney. Understanding the molecular response to injury in each segment should facilitate development of methods to accelerate repair after IR injury.

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Key Words: Hydroxyl radical, ischaemia-reperfusion, apoptosis, dimethylthiourea, allopurinol

INTRODUCTION

Oxygen-derived free radicals (OFR) formation during reoxygenation, and subsequent oxidative reactions such as lipid peroxidation, are implicated as causal factors in ischaemia-reperfusion (IR) injury (1). However, the relative contributions of OFR-generated oxidation to tissue injury versus that caused by other mechanisms during or after IR, such as ATP depletion or protease and phospholipase activation, are not known and may vary among tissues. Hydroxyl radicals ($^{\cdot}OH$) are thought to be the most reactive and injurious of the OFR. Once generated, they react with molecules and cell components in their immediate vicinity (2). Allopurinol, a known xanthine oxidase (XO) inhibitor, and dimethylthiourea (DMTU), an $^{\cdot}OH$ radical scavenger, have been widely used to assess IR injury attributable to OFR in different organs. In an vivo model of renal IR, it was demonstrated that DMTU, the $O_2^{\cdot -}$ scavenger superoxide dismutase (SOD) and allopurinol pretreatment each provided functional protection against injury (3). This study evaluated changes at 24, 48 and 72 hours post ischaemia, and reported little tubular injury in SOD treated kidneys. However, conflicting results for the usefulness of different antioxidants and scavengers have been reported for the kidney (4,5), suggesting that the significance of OFR in IR injury remains uncertain.

We have recently detected $^{\cdot}OH$ radicals during control perfusion which increased after IR (6,7). Both basal and increased formation of $^{\cdot}OH$ radicals during reperfusion after ischaemia were inhibited by the $^{\cdot}OH$ scavenger, DMTU. Although DMTU prevented both basal production and reperfusion-induced increases in $^{\cdot}OH$ production, it did not improve function during brief (3-15 min) reperfusion. In contrast, preteatment with DMTU or dimethylsulphoxide (DMSO) has been reported to reduce functional injury after 60 min reperfusion in vitro following 30 min renal artery clamping in vivo (8). The explanation for apparent prevention of $^{\cdot}OH$ production without functional protection in the scavenger-treated group is that in the latter the reperfusion interval was too short for improvement to be detected.

Renal ischaemia is the most common cause of acute renal failure (ARF), and results in defects in vascular, glomerular and tubular function and structure. The process of reperfusion after renal ischaemia in a surviving kidney or animal is associated with morphological damage primarily in the proximal tubules

and capillary basement membrane. Kidneys exposed only to acute ischaemia without recirculation do not show these abnormalities (9), although prolonged periods of severe ischaemia are known to cause cellular necrosis. The fact that reperfusion is necessary to end ischaemia and allow recovery adds to the central importance of the IR phenomenon. A comparison between altered morphological and functional parameters would be of assistance in estimating renal dysfunction and improvement on treatment. In a structural or morphological sense, ischaemia-induced renal cell injury has usually been attributed to necrosis, a passive or "accidental" form of cell death. The identification of the alternative type of cell death, apoptosis or programmed cell death, may provide new therapies involving modulation of this gene-driven form of cell death. However, apoptosis has not been identified after IR in the perfused kidney.

Overproduction of OFR, or a decrease in antioxidants, is known to be involved in the initiation of apoptotic cell death (10). Apoptosis has been described in several instances of renal disease involving ischaemic injury (11,12) including IR injury (13). An explanation for this specific death process in IR injury is burst generation of OFR during reperfusion (14). In the study by Schumer and colleagues (1992) (13), increased levels of apoptosis were descibed in tubular epithelial after 5 or 20 minutes ischaemia followed by 48 hours reperfusion, in vivo.

The present study assessed the effect of DMTU and allopurinol, on functional and structural damage associated with brief (15 min) or prolonged (60 min) reperfusion after a 20 min period of ischaemia in the isolated perfused rat kidney. A second aim was to document the extent of apoptosis in the acute phase of IR in this model, and to assess whether the antioxidants altered apoptotic cell numbers.

MATERIALS AND METHODS

Chemicals

Allopurinol was obtained from Sigma Chemical Co. (St. Louis, USA). Glutharaldehyde (TAAB, EM grade) was purchased from Probing and Structure (Townsville, Australia). DMTU was obtained from Aldrich Chemical Co. (Milwaukee, USA). All chemicals were of highest purity.

Isolated perfused rat kidney (IPRK) preparation

Experiments were performed on male Wistar rats weighing 250-350g with free access to standard food and water prior to treatment. The animals were anaesthetised by a single i.p. injection of 60 mg/kg sodium pentobarbital. Kidneys were isolated and perfused under carefully controlled conditions using a modification (15) of the method by Nishiitsutsuji - Uwo and coworkers. (1967)(16).

Briefly, the right renal artery was cannulated through the superior mesenteric artery immediately after i.v. injection of 500 U/kg sodium heparin. Perfusion pressure was continuously monitored within the renal artery using a Statham-type pressure gauge (model P23, Gould, UK) through a polyethylene line contained within the cannula perfusing the artery and calibrated for bovine serum albumin (6.7 g/100 ml), 5 mM D-glucose and essential amino acids. The initial volume of medium in the system was approximately 200 ml and the solution was gassed with 95% O_2 5% CO_2, bringing the pH to 7.4 Oxygen tension was measured in line by an oxygen electode attached to a blood gas analyser (PHM72 Radiometer, Copenhagen, Denmark).

The mean arterial pressure was monitored and held at 100 ± 20 mmHg using a process controller (model 2073, West Division, Gulton Industeries Inc., Schiller Park, USA) that controlled the speed of the peristaltic pump (Watson and Marlow model 501U; Smith and Nephew, Watson-Maelow, UK). Flow rate was measured by wide beam ultrasound using a Transonic Transonic T206 flow meter with an in line "cannulating" flow probe (SN22; Transonic Systems Inc., Ithaca, USA). Analog outputs from pressure, flow and oxygen meters were connected to separate channels of a chart recorder (Gould RS 3400, Gould Inc., Cleveland, USA). Mannitol (300 mg/kg i.v.) was injected and the right ureter was cannulated by a 2 cm length of 0.61 mm OD polyethylene tubing (ID 0.28 mm, Dural Plastics & Engineering, Auburn, Australia) connected to a 10 cm length of 0.96 mm polyethylene tubing (ID 0.58 mm).

Sample collection

Kidneys were perfused for a total of 75 or 120 min and renal function was monitored throughout in all groups using 14C-inulin clearance (glomerular filtration rate, GFR), renal vascular resistance (RVR) and fractional sodium excretion (FE_{Na}). For this purpose, 5 min urine collections were obtained after 25 min of experimental equilibration. Perfusate samples were taken at the mid-point of each urine collection period. The urinary clearances of 14C-inulin and sodium were calculated from their respective urine and perfusate concentration ratios. Sodium was measured by flame photometry (FLM3, Radiometer, Copenhagen, Denmark). The radioactiveity if 14C-inulin was counted in an LKB 1217 Rackbeta scintillation counter. Kidneys were accepted for inclusion only if the initial inulin clearance was greater than 0.5 ml/min/g kidney weight (calculated using the weight of the unperfused left kidney).

At the end of each experiment, kidneys were

perfused with 2.5% glutharaldehyde (TAAB, Probing and Structure, Australia) in phosphate-buffered saline (PBS: NaCl 8 g, KCl 0.2 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.2 g/l), using a 3-way tap. Perfusion fixation was performed for 2-3 min, until the kidney was pale, firm and completely fixed. The kidneys were then removed from the IPRKcircuit, halved transversely to the renal poles, and placed in fresh fixative for 2 hours.

Experimental protocol

The following groups of rats were studied:

1- Control : Kidneys were perfused for 75 or 120 min without supplementation or intervention and then fixed for histological examination (n=5);

2- Ischaemia-Reperfusion (IR): After 40 min baseline perfusion, ischaemia was induced for a total of 20 min by clamping off one arm of a Y piece. This stopped perfusion but allowed the perfusate to flow over the kidney maintaining temperature and hydration. Ischaemia was followed by 15 min or 60 min normoxic reperfusion and then fixation (n=5);

3- DMTU-IR: As for group 2 except that 15 mM DMTU was added to the perfusate 5 min before ischaemia followed by 15 min or 60 min normoxic reperfusion (n=5);

4- DMTU-Control: As for group 3 without ischaemia (n=3).

5- Allopurinol-IR: As for group 2 except that, allopurinol was injected (50 mg/kg i.v.) 10 min before arterial cannulation. A further 1mM allopurinol was added to the perfusate before induction of ischaemia followed by 15 min or 60 min normoxic reperfusio (n = 5).

6- Allopurinol-Control: As for group 5 without ischaemia (n=3).

Histology

Section were prepared form kidneys using routine histological methods. Section (3-4 μm) from paraffin-embedded transversely-sectioned kidneys were made and stained with haematoxylin and eosin (H&E) or periodic acid-schiff (PAS) for viewing by light microscopy.

Electron Microscopy

Small blocks (3 × 2mm length and breadth, and 1mm thick) of cortex and medulla from each kidney were placed in 0.1 M sodium cacodylate buffer, pH 7.2 (BDH Pty. Ltd., Melbourne, Australia). The tissues were postfixed in 1% aqueous osmium tetroxide, stained with saturated aqueous uranyl nitrate and then embedded in epoxy resin after routine dehydration and clearing procedures. Semi-thin section (1 μm thick) were cut on an LKB Ultratome V, and stained with toluidine blue for viewing by light microscopy. Ultrathin sections from the selected areas of semi-thin section were prepared for ultrastructural studies. Reynolds lead citrate (1-2 min) was used for staining the ultrathin sections, which were then examined using a JEOL 1200 EXII electron microscope (Tokyo, Japan).

Assessment of cell death

Paraffin and resin sections were viewed and photographed in a Leitz Orthoplan photomicroscope. Histological sections were labelled only with an identifying code number, and morphological examination was done "blind" to the treatment. The distinct morphological features of apoptosis and necrosis (17) were used to assess the presence of each form of cell death. Both apoptosis and necrosis have well-defined sequences of events (reviewed by Walker et al., 1988)(17). In apoptosis, there is condensation of the cell cytoplasm and nuclear chromatin (often against the nuclear envelope, often in crescentic masses), nuclear fragmentation, controlled blebbing of the affected cell into membrane-enclosed, structurally-preserved portions, termed apoptotic bodies, which are then often extruded into lumina of vessels, tubules and other luminal structures, or phagocytosed by adjacent viable cells. Necrosis involves cellular swelling, breakdown of cell and nuclear membranes, and although the nuclear chromatin may in some cases become condensed (during karyorhexis or pyknosis), it is distinctly different from chromatin changes seen in apoptosis.

Necrosis after ischaemia-reperfusion usually involved contiguous tubular epithelial cells, while apoptosis involved isolated cells. Quantitation of cell injury & death was graded semi-quantitatively for necrosis into 0 (no damage), + (up to 25%), ++ (26-50%) and +++ (more than 50%) damage. The incidence of apoptosis in individual cells was semi-qualitatively described. For each kidney, 10 × 20 magnification light microscope fields of superficial & deep cortex and inner & outer stripe of outer medulla were counted. Injury to different tubule segments was assessed using high power (×40 or × 100) microscopy.

Statistical analysis

For each time point, values are reported as mean ±SD for GFR, RVR and FE_{Na}. The Student's t-test comparisons were used to identify significant differences. $P < 0.05$ was assumed to be significant.

RESULTS

Structural assessment

Table 1 gives a semi-quantitative assessment of the structural alterations in each of the treatments.

Control without ischaemia (75 or 120 min perfusion)

Sections from these kidneys (group 1) showed normal histology. Some brush border and cellular vacuolation was seen, perfusion and fixation artefact. All cells were easily identifiable, glomeruli were normal, tubular lumina were patent. Neither apoptosis nor necrosis was seen.

DMTU and allopurinol controls (no ischaemia)

Sections from these kidneys (group 4 and group 6) were comparable with untreated controls. The glomeruli were normal. There was more of a tendency for brush border damage than was seen in untreated controls. Proximal convoluted tubule (PCT) inter and intracellular vasculation, vaculation in epithelial cells of the distal nephron, and some cast formation were also identified.

Ischaemia-reperfusion (Figs. 1A, 2A-D)

Details of the observed changes in group 2 kidneys are given in the legends to relevant figures and are summarised below. Glomeruli showed no changes detectable by light microscopy. In tubules, brush borders of the were attenuated, vaculoated, or shed (Figs. 1A, 2A and 2C). Epithelial cells of the PCT showed some nuclear changes, which were relative to the duration of reperfusion, increasing in intensity and frequency with longer (60 min) reperfusion time (Figs. 2A-D). Some cellular changes were similar to those described as reversible cell damage by Trump and coworkers, (1974)(19), the most notable of these changes being swelling of mitochondria without flocculent densisties. Frank necrosis was also identified, with grossly swollen mitochondria containing flocculent densities, and swelling and disruption of the nuclear membrane.

Loss of adhesion properties of epithelial cells is known to cause apoptosis (20). Over a longer experimental time, these apparently viable cells may become apoptotic. Mitosis was rarely seen. Morphological evidence of apoptosis was seen in several sections from both reperfusion times, but was not a prominent feature (Fig. 2B). Infiltrating monocytes or activated macrophages were occasionally identified. Dilatation of the tubular lumina, or collapse of tubules, was identified mixed forms of casts (hyaline, granular and pigmented) could be seen in proximal tubular lumina when loops of Helen, ascending limbs and distal convoluted tubules also showed cast formation (Fig. 2S). Some casts were proteinaceous (pink stain with PAS). The thick ascending limb (TAL) is the site of Tamm-Horsfall protein production, and the tubular casts have been reported elsewhere to contain this protein (21). Occasionally, epithelial cells that had been shed into the lumen were observed within the luminal casts, and showed signs of degradation (nuclear and cytoplasmic lysis, Fig. 1D). The TAL epithelial cells demonstrated nuclear pyknosis and some necrosis. Distal convoluted tubules were often packed with cast material, with squamous epithelial cells, and loss of damaged or apparently undamaged cells into the lumen. In the interstitium, there was no abnormal expansion or proliferation. Occasional neutrophils or leucocytes were identified. In vessels, little damage was apparent, with healhy endothelial cells lining the walls of the vessels. Occasional focal areas of necrosis in tubules were associated with damaged vessles.

Table1 : Morphological injury after 60 min reperfusion

	PCT	PST	TAL/O	TAL/I	TL	DT	CT
Control	+	+	+	+	0	+	0
		*	*	*			
Ischaemia reperfusion	++	+	+++	+++	+	+	+
	*	**	**	*		*	
DMTU-ischaemia	+	+	+	++	0	+	+
reperfusion	*	**	**	*		*	
Allopurinol ischaemia	++	+	++	++	0	+	+
reperfusion	*	*	*	*		*	

* Persence of luminal debris

Key :

0	no damage
+	up to 25%
++	26-50%
+++	> than 50%

PCT (proximal convoluted tubule), PST (proximal striaght tubule), TAL/O (outer stipe of the outer medulla thick ascending limb), TAL/I (inner stripe of the outer medulla thick ascending limb), TL (thin Limbs), DT (distal tubule), CT (collecting tubules in the inner medulla)

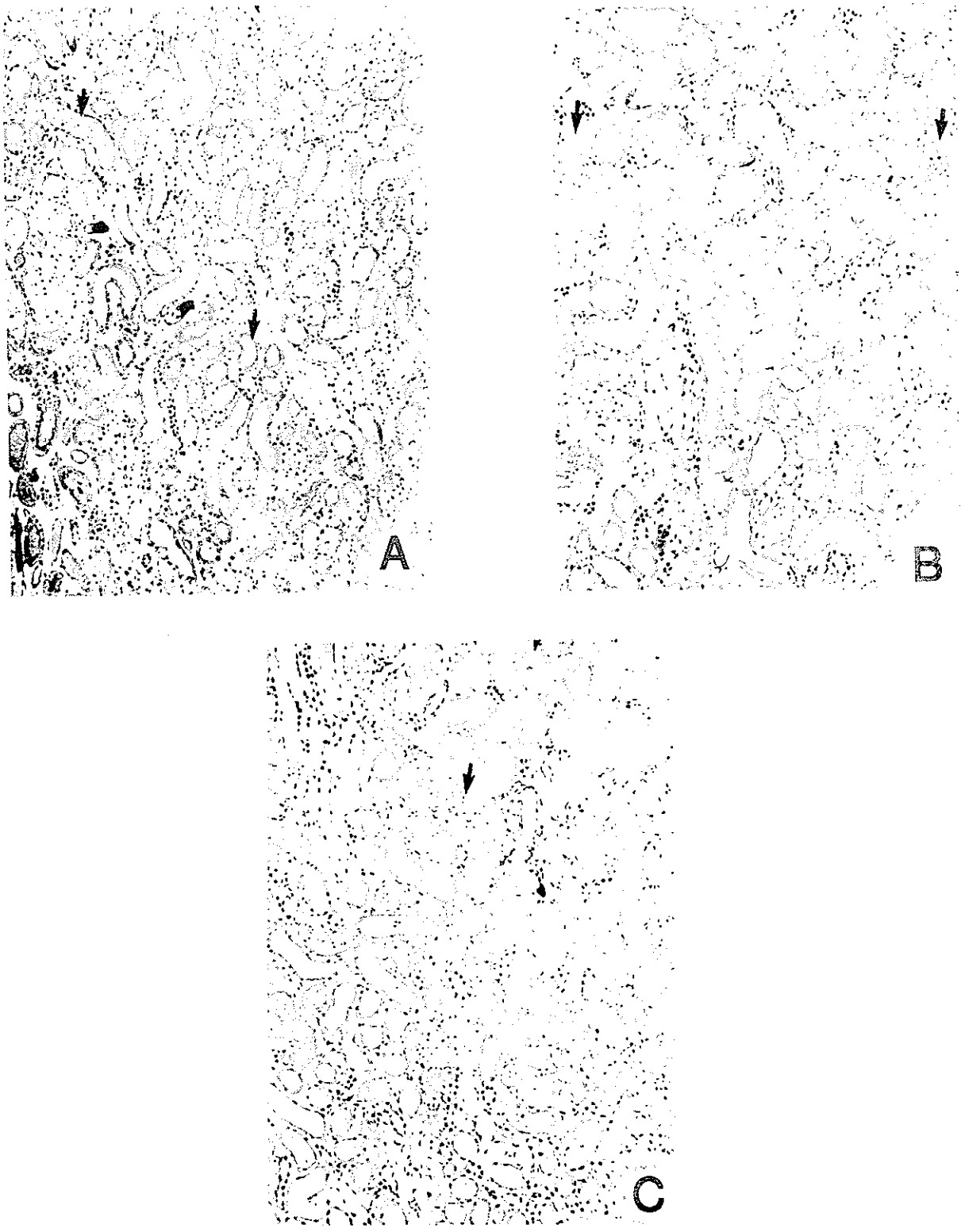

Fig. 1A, B and C. Photomicrographs of ischaemia-reperfusion injury with and without treatment, demonstrating features refers to in low power overview photomicrographs (Figs 2 and 3);

A. Outer medulla of an ischaemia- 60 min reperfused kidney, showing cellular blebs and casts in the lumina of many tubules (some examples are arrowed). As mentioned in the text, electron microscopy confirmed that the casts were in both the thick ascending limb and the S3 section of the proximal tubule.

B, C. Outer medulla of DMTU- (B) or allopurinol- (C) ischaemia- 60 min reperfused kidneys. Cellular blebs and cast material (arrows) were far less frequently observed especially in the DMTU treatment group.

Magnification A, B & C x 280.

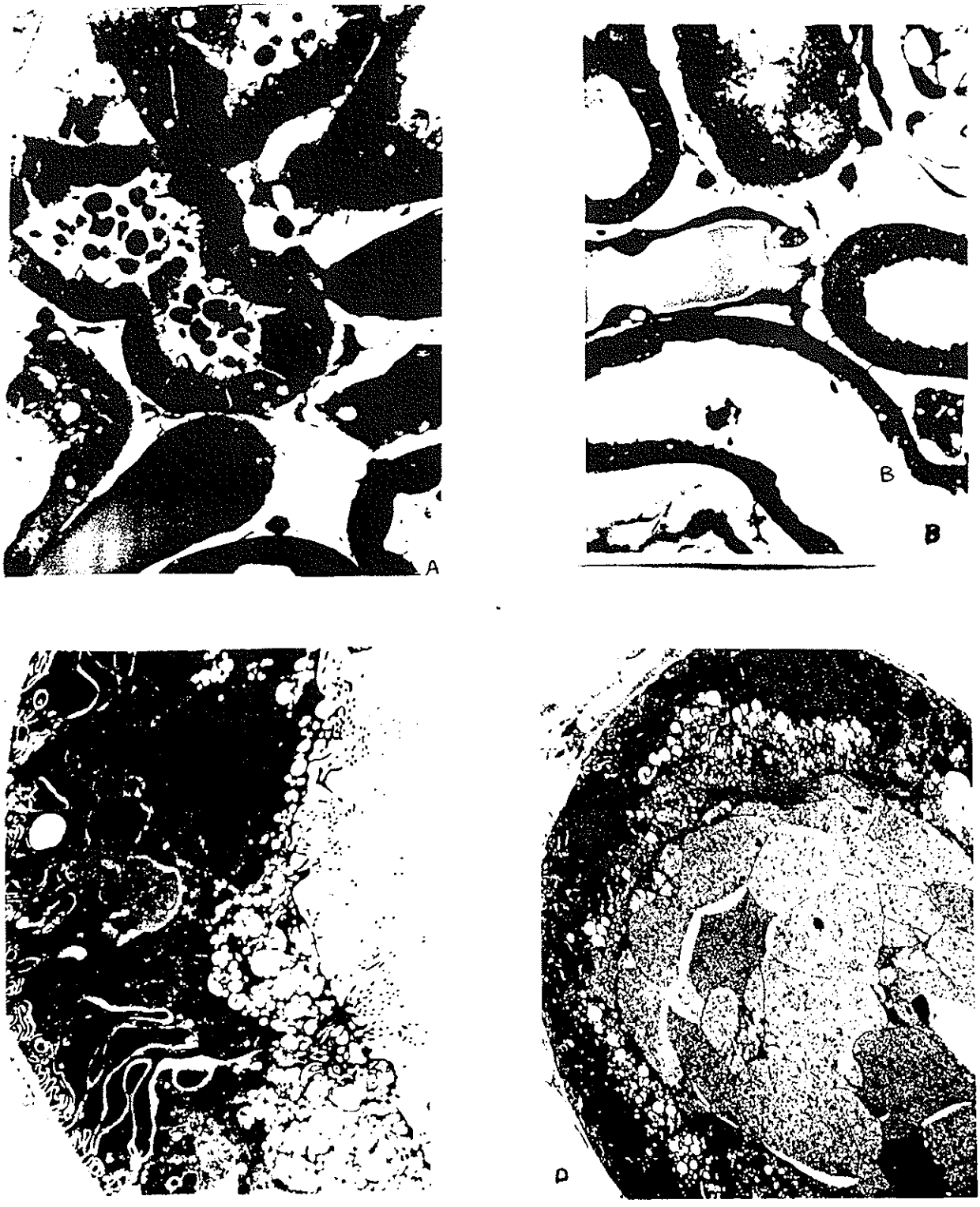

Fig. 2A, B, C and D. Ischaemia-reperfused kidneys.

A. Cast formation in the damaged tubules, showing proteinaceous material (eosinophilic). Tubules showed casts that often contained necrotic epithelial cells that had sloughed into the tubular lumen. Vacuolar cell swelling protrudes into the lumen (small arrows). Epithelial cell nuclei were occasionally pyknotic or lytic (large arrows).

B. The cell (arrowed) in the lumen shows condensed nuclear chromatin that appears in section to be closely marginated to a convoluted nuclear membrane. Such nuclear change is typical of apoptosis.

C. Cells show cytoplasmic vacuolation, evidence of lysosomal degradation and granular deposits that may be the result of breakdown in cell metabolism. Brush borders are attenuated or lost. Nucleus shows irregular clumping of the nuclear chromatin. These changes are indicative of cell injury.

D. Granular cast material in proximal tubules of ischaemia-reperfused kidney (large arrow), and evidence of lysosomal digestion perhaps as a result of autophagy visible in cytoplasm (curved arrows);

Magnification A x 400, B x 400, Cx4500, Dx5500.

DMTU- and allopurinol - ischaemia -reperfusion (Figs. 1 B, C, 3A, B)

There was evidence of reduced injury in both group 3 (DMTU-IR) and group 5 (allopurinol-IR) kidneys. Many cortical tubules were healthy, with some cast material (Fig. 1B, C). Some nuclear pyknosis and/or karyolysis was seen in the PCT epithelium, brush borders were flattened or sloughed into the lumina, and evidence of autolysis and endocytosis was found. Whole cells were seen in the lumina, and morphological evidence of apoptosis was seen in few sections in each treatment. The TAL epithelial cells again demonstrated nuclear pyknosis andsome necrosis, and proximal and distal tubular epithelium took on a squamous appearance. Interestitial damage was minimal, and mitosis was seen only occasionally, as in control sections.

Another difference between group 3 (DMTU-IR)-or group 5 (allopurinol-IR) kidneys and group 2 (IR) kidneys was the amount of cast material in the tubular lumen in the cortico-medullary zone. In all three groups (IR, DMTU- or allopurinol-IR) cast formation was easily identified after 15 min of repersufion. However, in the DMTU - and allopurinol-treated animals, 60 min reperfusion was associated with a marked reduction in cast material, suggesting improval in the patency of tubular lumina (Figs. 3A and 3B). Sections from both treatments showed development, in occasional tubular epithelial cells, of a homogenous, dark-staining material has been described by Kriz and coworkers, 1995 (22), in a study that included tubular damage after focal segmental glomerulosclerosis.

Functional assessment

Renal function was assessed from the GFR (Fig. 4, inulin clearance), fractional sodium excretion (Fig. 5, FENa) and renal vascular resistance (Fig. 6, RVR). Functional parameters were not significantly different amongst groups prior to ischaemia: Inulin clearance 0.57 ± 0.10 (mean ± SD, ml/min/g); FENa 0.05 ± 0.03 and RVR 2.2 ± 0.5 (mmHg. min/ml). Normalised values are demonstrated to highlight the difference between the groups after ischaemia-reperfusion.

During 15 min reperfusion renal function was markedly reduced in ischaemia - reperfused group as shown by the decrease in GFR (0.87± 0.18 vs 0.109 0.076, $p<0.001$) and by the increase in FE_{Na} (1.303± 0.47 vs 7.37 ± 4.34, $p<0.005$) and RVR (0.98±0.03 vs 1.18 ± 0.10, $P< 0.005$) compared with the control group ($n = 5$). Functional parameters were also different between ischaemia - reperfused kidneys (60 min reperfusion) and time matched controls: GFR, 0.72±0.20 vs 0.19 ± 0.15 ($P<0.005$); FE_{Na}, 1.85 ± 0.40 vs 5.54 ± 2.42 ($P<0.01$) and RVR, 0.98 ± 0.06 vs 1.18 ± 0.12 ($P<0.02$, $n=5$).

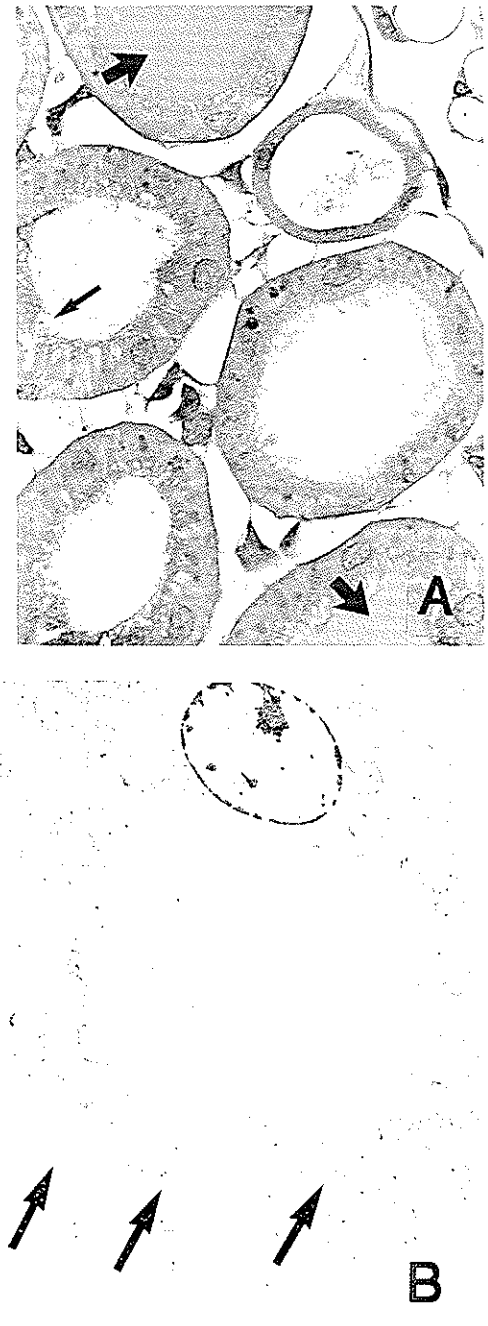

Fig. 3A and B. Ischaemia-reperfused kidneys treated with DMTU/ allopurinol.

A. An improvement in proximal tubular morphology was seen in an ischaemia- 60 min reperfused kidney after treatment. Some tubules were patent with normal brush borders. Some brush borders were lost (small arrow) and some cast material found (large arrows); B. In the inner zone of the medulla in an ischaemia- 60 min reperfused treated kidney, collecting tubules showed swollen mitochondria with few densities (arrows) but were otherwise viable;

Magnification A × 400, B × 3250.

GFR and FE_{Na} in the DMTU- and allopurinol -ischaemic groups were not significantly different from the ischaemic group but were consistently slightly better although not significantly different in allopurinol-ischaemic group than the ischaemic group (by ANOVA). The RVR at 60 min reperfusion in allopurinol-ischaemic group was significantly better than the ischaemic group 1.01 ± 0.08 vs 18 ± 0.12 ($P<0.03$, $n=5$).

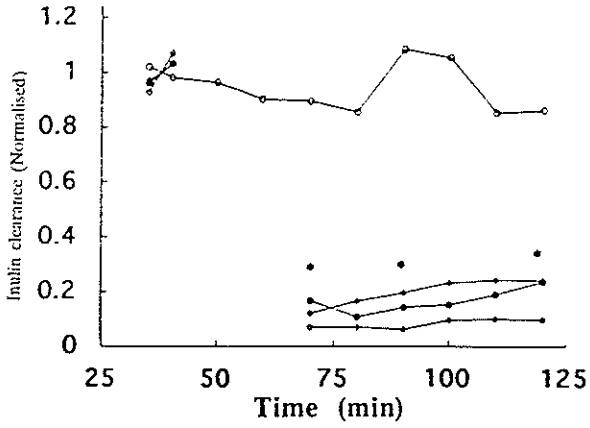

Fig. 4- Inulin clearance (GFR) in perfused rat kidney. Values represent the mean for each group. Key: (○) Control, (●) Ischaemia-reperfusion, (◇) DMTU-ischaemia- reperfusion, (◆) Allopurinol-ischaemia - reperfusion. Significance level: ** P< 0.001

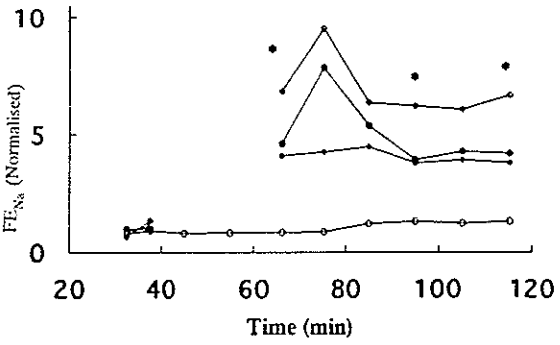

Fig. 5- Fractional excretion of sodium (FE_{Na}) in perfused rat kidney. Vlues represent the mean for each group. Key: (○) Control, (●) Ischaemia-reperfusion, (◇) DMTU-ischaemia- reperfusion, (◆) Allopurinol-ischaemia - reperfusion. Significance level: ** P< 0.005

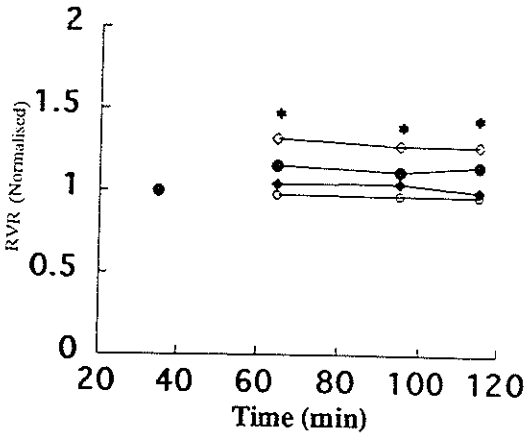

Fig. 6- Renal vascular resistance (RVR) in perfused rat kidney. Values represent the mean for each group. Key: (○) Control, (●) Ischaemia-reperfusion, (◇) DMTU-ischaemia- reperfusion, (◆) Allopurinol-ischaemia - reperfusion. Significance level: ** P< 0.01

DISCUSSION

Tubular obstruction and afferent arteriolar constriction result in reduced glomerular filtration rate, increased renal vascular resistance and elevation of intratubular pressure. These events have been widely implicated as possible aetiologic mechanisms of IR injury, leading to the death of renal cells(23). In most publications renal cell death associated with the syndrome of acute renal failure, is generically termed "acute tubular necrosis", but another form of cell death, namely apoptosis or programmed cell death may also be involved. Cellular injury occurring in IR results from the interaction of multiple processes, including damage by OFR, especially in the absence or malfunction of appropriate antioxidants. One important recent observeation is that OFR can be involved in the induction of apoptosis(10).

The present study aimed to assess the relationship between the functional impairment found in the previous studies and changes in renal morphology after IR in IPRK. The capacity for DMTU, a known 'OH radical scavenger and allopurinol, a xanthine oxidase

inhibitor to improve IR-induced functional impairment and/or structural damage was also assessed. DMTU has been shown to improve GFR in gentamicin-mediated nephropathy (24) and protects myocardial tissues against lipid peroxidation by OFR (25). Allopurinol is rapidly converted by xanthine oxidase to its major metabolite oxypurinol which inhibitis the XO-mediated conversion of hypoxanthine to xanthine and xanthine to uric acid (25-6). Allopurinol has been shown to be effective in reducing protein loss in IPRK (27), in reducing OFR reperfusion injury in transplanted kidneys (28), in ischaemic rat liver (29) and in myocardial IR injury (30). Similarly, oxypurinol is reported to minimise intratubular cast formation in renal IR injury in vivo (31).

Allopurinol and oxypurinol also act as direct scavengers of 'OH radicals. The attack of 'OH radicals on allopurinol produces oxypurinol which is itself a better direct 'OH scavenger than allopurinol (32).

Direct scavenging of OFR by allopurinol is supported by its protection against IR injury in rabbit myocardium, which does not have XO activity (33). This suggests an alternative therapeutic role for allopurinol in IR injury (25-6). However, conflicting results have also been reported (25). For example, treatment with oxypurinol did not improve inulin clearance in the early postischaemic period in the kidney (31). There are suggestions that allopurinol may need multiple dosing to be useful (30), and further research into the potential beneficial action of this drug is needed.

Using direct detection of OFR by chemical or paramagnetic spin traps, we have demonstrated previously that DMTU reduces the 'OH radical generation after IR in perfused kidneys without preventing the functional impairment seen after a short (15 min) period of reperfusion (6,7). Depending on the duration of ischaemia, the post-ischaemic kidney may be able to restore structure and function completely (34) after a sufficient period of reperfusion. Therefore in this study two different periods of reperfusion, 15 and 60 min, were chosen to assess whether longer periods of reperfusion would allow the putative protection of parameters of function and structure by DMTU and allopurinol to be detected (8).

The structural damage identified after IR involved mainly the cortico-medullary and outer medullary regions. Cell swelling, nuclear swelling and some cases of obvious necrosis were seen. Loss of brush borders in cells of the proximal convoluted tubules was common, along with luminal debris, and cast formation mainly seen in the TAL. Some apoptosis was identified using morphological parameters in the proximal tubular cells in IR-treated animals, but the low frequency of apoptosis did not suggest a significant role for this type of cell death after IR. DNA fragmentation was observed in the distal tubule, medullary TAL, and collecting ducts. The relationship of DNA fragmentation to actual apoptosis is uncertain as some fragmentation can occur without progression to apoptosis.

Apoptosis is often underscored because of its occurrence as an individual (compared with confluent) cell process, and its short duration (35). This has led to the development of biochemical markers of apoptosis to try and identify apoptosis more accurately. However, there is controversy regarding the most useful markers for apoptosis. Kerr et al. (1995) (36) regard morphological criteria as essential for identification of apoptosis. Wyllie et al. (1984) (35) demonstrated that the apoptotic process is rapid, having 4h from initiation to phagocytosis. The timing used in the present model may not have been sufficient for the development of extensive apoptosis as identified morphologically. The in situ end labelling that was identified may indicate that early DNA fragmentation has occurred prior to morphological changes of apoptosis. Alternatively, the end-labelling found may merely indicate DNA damage, including single-stranded damage, that does not proceed to apoptosis but is repairable. Extended perfusion will be required to determine if apoptosis actually does occur in the population of cells of the TAL and distal nephron.

In some sections epithelial cells had been shed into the lumen. These cells include damaged and apparently undamaged cells. Racusen and coworkers (1991) (37) observed that the shed cells are mostly reversibly injured cells that can be grown in culture. This observation opened up the study of adhesion molecules in the kidney and has led to the study of factors that can inhibit initial detachment of these cells from the basement membrane or prevent luminal cell aggregation and tubular obstruction (38).

Neither DMTU nor allopurinol significantly improved the IR-induced impairment in renal function after both short or long durations of reperfusion, although function in the allopurinol-treated group was slightly better than the other groups. This result is supported to some extent by the morphological study, which showed that allopurinol treatment caused only a modest improvement in pathological feature of injury. The most notable change with DMTU and allopurinol treatments was the reduction in the amount of cast material in the tubular lumina after 60 min reperfusion. Increasing the patency of the tubules should have long term benefits in improving recovery of renal structure and function.

Apoptotic bodies or in situ end-labelled cells were observed in this model of IR injury, despite the relatively short period of reperfusion. Although morphological evidence of apoptosis was infrequent, ISEL was high and was decreased in the inner

medullary collecting tubules with DMTU and allopurinol treatment. This novel observation is potentially important. It suggests that the OFR scavengers may be acting to limit induction of apoptosis in these sites. Another interesting finding was the detection of monocytes in the damaged tissue. As there are no white cells in the medium, these monocytes apper to have come from local transformation of resting cells already in the interstitium when perfusion was commenced. Irrespective of their origin, the presence of monocytes in the interstitium suggests they may play a role in this model of IR injury.

In this acute study of IR injury, most of the cell damage was characteristic of necrosis. Treatment with either allopurinol or DMTU had limited effect on changes in structure or function, although over a longer time frame or with multiple dosing either could still be beneficial, particularly since there was reduced ISEL for DNA fragmentation in inner and outer medullary zones after both treatments. The results reported in this study shows a trend for improvement in morphology after both treatments. The results emphasise the individual susceptibility of nephron segments to injury and suggest that, if identified, modifying the molecular or biochemical controls acting in each segment could be useful in recovery or reducing the damage to the kidney caused during IR.

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