Mesna: a novel renoprotective antioxidant in ischaemic acute renal failure

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Abstract

Background. Reactive oxygen species (ROS) play a key role in renal ischaemia–reperfusion injury. After establishing the in vitro anti-oxidative potential of mesna, a sulfhydryl-containing compound, its effect on kidney function and morphology in a rat model of ischaemic acute renal failure (ARF) was examined.

Methods. Mesna (180 mg/kg) was administered at different time points relative to ischaemia and/or reperfusion onset. Kidney function was assessed by glomerular filtration rate (GFR) and fractional sodium excretion (FENa) before a 45-min period of unilateral renal artery clamping and following 90 min of reperfusion. Mesna was administered by bolus, 30 min before the induction of ischaemia, 5 min before ischaemia, 5 min before reperfusion, and 5 min after the onset of reperfusion.

Results. Mesna improved function of the ischaemic kidney at each administration. When mesna was administered 5 min before the onset of reperfusion, GFR reached 90–100% of its pre ischaemic value and FE Na was improved by 75%. The beneficial effect of mesna was also demonstrated by light and electron microscopy. Kidneys treated with mesna 5 min before reperfusion resembled ischaemic non-reperfused kidneys and showed subtle morphological and ultrastructural changes compared with ischaemic–reperfused kidneys. Mesna had no haemodynamic effect on renal blood flow and did not induce any osmotic diuresis.

Conclusions. We suggest that mesna acts as an antioxidant. Its antioxidant potential together with optimal protection achieved when administered 5 min before reperfusion, supports the conclusion that mesna scavenges ROS generated at the onset of reperfusion, thus diminishing reperfusion injury and organ damage.

Keywords: acute renal failure; antioxidant; ischaemia–reperfusion; mesna; reactive oxygen species; renal blood flow; renal morphology

Introduction

Renal ischaemia causes a series of cellular events, which occasionally lead to organ failure depending on the duration of oxygen deprivation. It has been assumed that reperfusion, if started before irreversible damage to the kidney occurs, could limit the insult to the organ. Contrary to such expectations, it has been reported that reperfusion can actually aggravate renal damage [1–3]. Reactive oxygen species (ROS) play a key role in this process [4,5]. These species may be generated in the ischaemic kidney at the onset of reperfusion, when the oxygen supply is renewed [6]. Alternatively, it has been proposed that polymorphonuclear leukocytes (PMNs) that are activated during ischaemia, presumably by cytokines, enter the kidney at the onset of reperfusion, causing tissue damage by releasing ROS [4–8].

Irrespective of the origin of ROS, one would anticipate that treatment with antioxidants would minimize ischaemia–reperfusion damage. However, treatment with antioxidants has produced only moderate success [9–12]. For example, the thiol-containing amino acid taurine reduced the ischaemic damage caused by PMNs in the ischaemic-reperfused heart. Addition of the antioxidant taurine to the perfusate, improved external heart work from 30% to 60% [12]. In a rat model of ischaemic acute renal failure, the antioxidant probucol, given for 2 weeks prior to ischaemia, significantly improved single-nephron glomerular filtration rate (SNGFR) from 30% in the ischaemic kidney to 62% in the treated kidney [10]. Treatment with superoxide dismutase (SOD) partially lowered plasma creatinine after 24 h of reperfusion following 60 min of renal ischaemia in the rat [11]. The
limited success of antioxidant therapy in preventing ischaemia–reperfusion damage may be explained by: (i) the inability of the antioxidant to access the intracellular environment due to its large molecular size or charge, (ii) the inadequate timing and route of administration of the antioxidant, and (iii) an ineffective intra- or extracellular concentration.

Mesna (sodium-2-mercaptoethane sulphonate) is used clinically to prevent the nephrotoxicity and urinary tract toxicity caused by cyclophosphamides such as ifosfamide [13]. More specifically, Schoenike and Dana [14] reported that the metabolites of ifosfamide, acrolein and 4-hydroxyifosfamide, are highly reactive compounds and responsible for the observed tubular and interstitial renal tissue damage following its administration. In addition, Skinner and Sharkey [13] noted that an intravenous bolus of mesna was more effective than a continuous infusion in reducing cyclophosphamide-induced tissue damage. Mesna itself is a small molecule and by virtue of its sulfhydryl (SH) group, has the potential to scavenge ROS [15,16]. When administered intravenously, mesna is auto-oxidized in blood to its unreactive disulphide form, dimesna [13,14]. Dimesna is taken up by renal tubular cells and reduced back to mesna by intracellular glutathione. Both mesna and dimesna are cleared from the vascular compartment by glomerular filtration, with a plasma half-life of 0.36 h. Twenty minutes after mesna administration, the greatest tissue concentration was found in the kidneys [17].

Due to the small molecular size of mesna, its ability to concentrate in the kidneys, and its antioxidant potential, it may be useful in minimizing renal ischaemia–reperfusion injury. Hence, the aims of our study were (i) to evaluate the ability of mesna to ameliorate kidney function and (ii) to determine the most appropriate time at which to administer mesna in order to attain the best improvement in renal function in a rat model of ischaemia followed by 90 min reperfusion.

To achieve these aims, three different series of experiments were performed. In the first series, kidney function was evaluated by determination of glomerular filtration rate (GFR) and fractional sodium excretion (FE Na) in the ischaemic and non-ischaemic kidneys with and without mesna treatment given at various time points. In a second series of experiments, the effect of mesna on renal blood flow (RBF) was assessed in order to include or exclude haemodynamic effects induced by the drug. In a third series of experiments, changes in kidney morphology were characterized at the end of the ischaemic period and after 90 min of reperfusion, in the absence of and following administration of mesna.

Subjects and methods

Animals and surgical procedure

We used an anaesthetized rat model of unilateral renal artery occlusion for the study of ischaemia–reperfusion injury. All procedures and experimental protocols were approved by the Committee for the Supervision of Animal Experiments, Western Galilee Hospital, Nahariya, Israel.

Female Sprague–Dawley rats, weighing 200–300 g were used. The surgical procedure has been previously described [18]. Briefly, the animals were anaesthetized by intraperitoneal injection using a combination of 10% chloral hydrate, 200 mg/kg (Merck, Darmstadt, Germany), and sodium pentobarbitone, 16 mg/kg (CTS Chemical Industries, Kiriat Malachi, Israel), and placed on a temperature-regulated table (37°C). After placement of an intratracheal tube, a cannula was inserted into the carotid artery for continuous blood pressure monitoring and blood-sample withdrawals. Another cannula was inserted into the jugular vein for intravenous administration of saline, inulin (Sigma, St Louis, MO, USA), and mesna (Asta Pharma AG, Frankfurt am Main, Germany). Saline containing inulin (27.5 mg/h) was infused intravenously at a rate of 4 ml/h to compensate for the blood withdrawn during the experiment. Cannulation of the left ureter and the urinary bladder enabled separate urine collections from each kidney. Animals were allowed to equilibrate for 30 min as assessed by steady urinary flow from both kidneys. The next 30 min served as a baseline period, during which the animals received saline and inulin only. Forty-five minutes of left unilateral renal artery occlusion, the ischaemic period, was followed by 90 min of reperfusion. Urine samples were obtained from each kidney separately every 30 min throughout the experimental period (Figure 1). At the midpoint of each urine collection, a blood sample (0.3 ml) was withdrawn. Serum and urine samples were kept at −20°C until analysed (maximum 1 month) for inulin and sodium concentrations.

Protocol I. Effect of mesna on kidney function

Forty-seven rats were randomly divided into five groups according to the different timing of mesna administration. All groups (except the control group) received a bolus of 180 mg/kg mesna, followed by a continuous infusion of 30 mg/kg/h for the entire duration of the experiment. This dose of 180 mg/kg was chosen after performing preliminary experiments in which lower doses of mesna were used. The 180-mg/kg dose was not lethal to the rats and appeared to be the most effective in ameliorating kidney function after ischaemia and reperfusion.

The study groups comprised:

(i) Control group (n=11) in which the left kidney was subjected to 45 min of ischaemia followed by 90 min of reperfusion;

(ii) 30 min pre-ischaemia group (n=10) that received an intravenous bolus of mesna 30 min prior to the onset of ischaemia;

Fig. 1. Experimental procedure.
(iii) 5 min pre-ischaemia group \((n=8)\) a group that received an intravenous bolus of mesna 5 min prior to the onset of ischaemia;
(iv) 5 min pre-reperfusion group \((n=10)\) that received an intravenous bolus of mesna 5 min before the onset of reperfusion;
(v) 5 min post-reperfusion group \((n=8)\) that received an intravenous bolus of mesna 5 min after the onset of reperfusion.

To assess the effect of mesna on kidney function, GFR and \(\text{FE}_{\text{Na}}\) were measured for each kidney separately for each period. GFR was determined by inulin clearance by assaying inulin concentrations in urine and plasma samples using anthrone method [19]. \(\text{FE}_{\text{Na}}\) was calculated from urine and plasma sodium concentrations measured by flame photometry (Instrumentation Laboratory 943) and divided by GFR. Kidney function obtained from the right kidney of each animal served as reference for each individual rat.

Protocol 2. Effect of mesna on renal blood flow (RBF)

Twelve rats were randomly divided into two groups in which RBF was measured:

(i) Control group, comprising six rats in which the left kidney was subjected to 45 min of ischaemia, followed by 60 min of reperfusion (no mesna given).
(ii) Mesna group, comprising six rats that received mesna (180 mg/kg) as an intravenous bolus 30 min prior to the onset of ischaemia, followed by a continuous infusion of 30 mg/kg/h for 60 min during reperfusion.

RBF was measured directly using an ultrasonic flowmeter (Transonic Systems Inc. T206, Ithaca NY, USA) connected to a transducer positioned around the left renal artery, as we have previously described [20]. Briefly, the animals were anaesthetized as described above and placed on a heated surgical table. After cannulation of the carotid artery and the jugular vein, the left renal artery was exposed and an ultrasonic probe was positioned around the renal artery. Mean arterial pressure (MAP) and RBF were measured continuously. Readings were recorded every 3 s during the experiment. In the control group, RBF was monitored for 30 min before the induction of ischaemia and for 60 min of the reperfusion period. As the absolute values of RBF, determined before the beginning of the experiment, differed slightly from one animal to another (between 6 ml/min and 10 ml/min), RBF values for each animal are expressed as the percentage from RBF at the beginning of the experiment (zero time) which is 100%. RBF data are presented as the group mean \(\pm\) SD of readings extracted from the recorded readings every 5 min.

Protocol 3. Effect of mesna on kidney morphology

Renal morphology was evaluated in left ischaemic kidneys harvested from four different experimental groups of rats:

(i) Control ischaemia rats, in which the left kidney was subjected to 45 min of ischaemia;
(ii) Control ischaemia–reperfusion rats, in which the left kidney was subjected to 45 min of ischaemia followed by 90 min reperfusion;
(iii) Mesna-treated ischaemia–reperfusion rats, in which the left kidney was subjected to 45 min of ischaemia followed by 90 min reperfusion, with the rats receiving mesna 5 min before the onset of reperfusion.
(iv) Sham-operated rats that underwent the entire surgical procedure without renal artery clamping and that were not treated with mesna.

Kidneys were harvested for light and electron microscopic evaluation. For light microscopy, kidney sections were fixed in 4% formalin and embedded in paraffin. Sections (5 \(\mu\)m) were stained with haematoxylin and eosin and periodic acid–Schiff (PAS) and examined under a light microscope. Renal morphology was evaluated by an independent observer and assessed with respect to disruption of renal architecture, focusing on the tubular morphology.

For electron microscopy, slices of the kidney cortices were fixed by immersion in 1% glutaraldehyde (GA) in phosphate-buffered saline (PBS), pH 7.4 at a temperature of 4°C for 1 h and then washed in PBS. Blocks of 1 mm\(^3\) of the GA-fixed kidney slices were post-fixed with 1% OsO\(_4\) in veronal-acetate buffer, pH 7.4 for 1 h at 4°C, dehydrated in ethanol and propylene oxide, and embedded in Araldite (Polysciences, Washington, Pennsylvania). Examination of the sections was carried out using a JEM 100B (JHOL, Tokyo, Japan) electron microscope at 80 kV.

In vitro activity of mesna as an antioxidant

To show that mesna has a direct scavenging effect on ROS, titration of its SH groups was carried out before and after incubation with superoxide and hydrogen peroxide. Mesna (1.0 mmol/l) was incubated with either a cell-free superoxide-generating system (xanthine (0.15 mmol/l, Sigma Chemical Co., St Louis, MO, USA)xanthine oxidase (XO) grade III, from buttermilk (0.017 U/ml, Sigma)) or with 100 mmol/l hydrogen peroxide, in 0.1 mol/l phosphate buffer (pH 7.5) containing 5 mmol/l EDTA, for 30 min at 37°C. When H\(_2\)O\(_2\) was used, before the titration of SH groups, the excess was decomposed with catalase 2 U/ml (Sigma). Determination in the range of 1–25 \(\mu\)mol/l SH groups of mesna was carried out by 2.0 mmol/l 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) monitored spectrophotometrically at 412 nm.

Data analysis

Data collected from protocols 1 and 2 are expressed as mean \(\pm\) SEM and mean \(\pm\) SD. Significance of the difference between the study parameters were estimated by one-way analysis of variance (ANOVA) using a Tukey–Kramer multiple comparisons post-test. Statistical significance was set at 5% level.

Results

GFR

Table 1 and Figure 2 summarize the changes in GFR following mesna administration in all groups. GFR values for all 30-min periods of urine collection throughout the experiment are shown in Table 1. In all mesna-treated groups during reperfusion, there is
Table 1. GFR (ml/min) as a function of the timing of mesna administration

<table>
<thead>
<tr>
<th>Period</th>
<th>Group</th>
<th>Baseline 30 min</th>
<th>30 min pre ischaemia</th>
<th>Ischaemia 45 min</th>
<th>60 min reperfusion</th>
<th>90 min reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Control</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>30 min pre-ischaemia</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>5 min pre-ischaemia</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>5 min pre-reperfusion</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>5 min post-reperfusion</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

GFR (ml/min) of experimental groups mean ± SEM, in the control right kidney vs the ischaemic left one. *P < 0.05; **P < 0.01 when compared to control group in the same period.

![Graph showing GFR (ml/min) after 90 min reperfusion](image)

**Fig. 2.** Effect of mesna administered at various times on GFR. Mean GFR values ± SEM (ml/min) for both kidneys of the experimental groups during the last 30 min of reperfusion are depicted. No ischaemic insult was applied to the right control kidney; 45 min of ischaemia followed by 90 min reperfusion were applied to the left kidney. *P < 0.05 vs the left kidney of the control group, **P < 0.01 vs the left kidney of the control group.

a slight, non-significant increase in GFR of the right (control) kidneys compared with baseline values. Mesna at all times administered, improved GFR in the ischaemic kidney at 60 and 90 min of reperfusion periods. Although the GFR at 90 min is higher than at 60 min, the increase in GFR from 60 to 90 min was not significant. To highlight the importance of mesna administration timing on kidney function, we chose to present GFR values only after 90 min, for both kidneys (Figure 2). The best amelioration in the GFR was achieved when mesna was administered 5 min before reperfusion. However, when mesna was given 5 min after the onset of reperfusion, its beneficial effect, although still significantly better than that seen in the control ischaemic–reperfused group, was reduced compared with the group that received it 5 min before reperfusion.

**FE$_{Na}$**

Table 2 and Figure 3 summarize the changes in FE$_{Na}$ following mesna administration in all groups. FE$_{Na}$ values for all 30-min periods throughout the experiment are given in Table 2. Mesna administered at all times improved FE$_{Na}$ when measured 90 min after reperfusion. A significant deterioration occurred in the FE$_{Na}$ of the control group between 60 and 90 min of reperfusion (Table 2, e vs f); the worst FE$_{Na}$ concentration occurred after 90 min. Compared with the lowest FE$_{Na}$ value, mesna induced a significant...
Table 2. FE_{Na} (%) as a function of the timing of mesna administration

<table>
<thead>
<tr>
<th>Period</th>
<th>Baseline 30 min</th>
<th>30 min pre ischaemia</th>
<th>Ischaemia 45 min</th>
<th>60 min reperfusion</th>
<th>90 min reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Control</td>
<td>0.9 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>1.7 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>30 min pre-ischaemia</td>
<td>0.5 ± 0.1^a^</td>
<td>0.6 ± 0.2^b^</td>
<td>1.9 ± 0.5^d^</td>
<td>2.0 ± 0.5^d^</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>5 min pre-ischaemia</td>
<td>0.2 ± 0.02</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.8</td>
<td>1.0 ± 0.3</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>5 min pre-reperfusion</td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>5 min post-reperfusion</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>2.0 ± 0.4</td>
</tr>
</tbody>
</table>

FE_{Na} of experimental groups mean ± SEM. in the control right kidney vs the ischaemic left one. *P ≤ 0.05; **P ≤ 0.01 when compared to control group in the same period. a vs b, P ≤ 0.01; c vs d, P ≤ 0.01; e vs f, P ≤ 0.05.

![Graph](image)

Fig. 3. Effect of mesna administered at various times on FE_{Na}. Mean FE_{Na} values ± SEM (%) for both kidneys of the experimental groups during the last 30 min of reperfusion are shown. No ischaemic insult was applied to the right control kidney; 45 min of ischaemia followed by 90 min reperfusion were applied to the left kidney. *P ≤ 0.05 vs the left kidney of the control group, **P ≤ 0.01 vs the left kidney of the control group.

improvement in FE_{Na} for all treated groups, with the greatest amelioration occurring when mesna was administered 5 min before reperfusion (Figure 3).

As mesna is a sodium salt, induced solute or osmotic protective effect of mesna can be ruled out by two finding:

(i) A significant increase of FE_{Na} was observed before induction of ischaemia, with no parallel significant increase in GFR in the same experimental group (Table 2, a vs b and c vs d), reflecting a normal physiological response to sodium load. In addition, bolus administration mesna had no effect on blood osmolarity (340 mOsmol before ischaemia and 340 mOsmol during reperfusion).

(ii) The beneficial effect of mesna was not a result of salt loading: in a control group that received a bolus of NaCl solution at the same molar concentration of mesna, no effect on renal function was detected after 90 min of reperfusion (data not shown).

RBF

As seen in Figure 4, RBF in the control group and the mesna group was similar. Mesna administration had no effect on RBF. Ischaemia, as expected, caused a cessation of RBF. RBF restoration started with the onset of reperfusion, reached 80% of its pre-ischaemic value within 15 min, and RBF was fully restored within 50 min, irrespective of mesna administration. As there were no further changes in RBF after 50 min, we do not show RBF beyond 60 min. MAP was 98–117 mmHg throughout the experiment, with and without mesna.

Light microscopy

In the control ischaemic-reperfused kidney, severe hydropic degeneration with a loose brush border was
observed in the proximal tubules (Figure 5A). In the ischaemic–reperfusion kidney treated with mesna 5 min before reperfusion, mild hydropic changes were seen and the brush border was almost intact in the proximal tubule (Figure 5B). No disruptions in the architecture of the proximal convoluted tubules were observed in the sham-operated kidney (Figure 5C).

**EM**

Figure 6 is an electronmicrograph of sham-operated kidneys showing intact tubules with mitochondria oriented perpendicularly to the basement membrane. In contrast, control ischaemia rats demonstrate tubular necrosis as well as blebbing of the plasma membrane. Mitochondria are damaged and demonstrate tiny flocculent densities (Figure 7). In control ischaemia–reperfusion rats exposed to ischaemia and 90 min reperfusion, there is a loss of normal tubular structure with detachment of the tubular cell from the basement membrane and disruption of the tubular basolateral membrane. Microvilli are shed into the tubule lumina. Numerous blebs and vacuoles of various sizes are present throughout the cell. The mitochondria are swollen with abnormal cristae and contain tiny flocculent densities (Figure 8). These changes are more profound than those observed in kidneys that underwent only ischaemia. In mesna-treated ischaemia–reperfusion the tubular cells are damaged, although less so than the non-treated ischaemia–reperfusion kidneys. The tubular basement membrane and basolateral membrane are intact and several vacuoles of varying size are observed. There are normal and swollen mitochondria, similar to the control ischaemic non-reperfused kidney (Figure 9).
**In vitro activity of mesna as an antioxidant**

Superoxide and hydrogen peroxide are representative species of the main ROS, generated due to ischaemia and reperfusion [7,8]. *In-vitro* incubation of \( \text{H}_2\text{O}_2 \) or superoxide with mesna caused 100% or 30–40% disappearance of the SH groups respectively. Hence, the antioxidant effect of mesna is possibly due to a direct scavenging effect on superoxide and hydrogen peroxide, by oxidation of SH groups.

**Discussion**

The data presented in this study show that mesna preserves renal function and kidney morphology following ischaemia–reperfusion. When mesna was administered 5 min before the onset of reperfusion, GFR reached 90–100% of its pre-ischaemic values and \( \text{FE}_{\text{Na}} \) was improved by 75%. Since ischaemia–reperfusion injury is mediated by ROS, the mechanism of action of mesna may be linked to its antioxidant potential. Other mechanisms that could explain the beneficial role of mesna, such as haemodynamic changes, osmotic diuresis, or natriuretic properties have been ruled out. The delay in tubular function recovery after 90 minutes of reperfusion, in contrast to the complete recovery of GFR, may reflect sodium loading.

Although mesna was effective at all times administered, the most beneficial effect was obtained when given in a bolus, just before the onset of reperfusion. This timing, and the knowledge that ischaemia–reperfusion injury is mediated by ROS, confirms the idea that ROS formation occurs just at the onset of reperfusion, and suggests that the beneficial effect of mesna is mediated by preventing ROS-induced damage rather than by correction of renal function.

The involvement of ROS in ischaemia–reperfusion injury to the kidney and other organs is widely accepted. The main reported sources for ROS production during ischaemia–reperfusion are endothelial cells, which have hypoxanthine/xanthine oxidase system, and activated neutrophils [21]. The formation of these injurious species at the onset of reperfusion is solely dependent on a preceding ischaemic, anaerobic period [5]. However, as it is impossible to measure organ function during ischaemia, very few studies, mainly intestine models [22], could prove this assumption. It has been shown that acute renal ischaemic injury induces marked structural changes in epithelial cells, especially in the proximal tubule [23]. Alteration of the cytoskeleton, disruption of normal cell–matrix interaction, and shedding of the apical microvilli into the tubular lumen lead to the inability of renal epithelium to maintain polarity and normal renal function. Reperfusion undoubtedly augments tissue injury. Our electron microscopy sections support the
Fig. 7. Electron micrograph of a proximal tubule from a control rat exposed to ischaemia only (×4000).

Fig. 8. Electron micrograph of a proximal tubule from a control ischaemia–reperfusion rat (×2700).
premise that ischaemia alone causes only mild tissue injury, while reperfusion is the culprit for the more severe tubular damage.

Various ROS scavengers have been used in different studies with conflicting results [10,11]. These contrasting data can be explained by varying tissue concentrations of the scavenger at the time of ROS generation due to differences in chemical and physical properties of the scavengers used, such as molecular weight and charge, in addition to the timing and mode of administration.

The scavenger used in this study, mesna, a small molecule containing an SH group, has an antioxidant potential as shown by our in vitro study. Our results show that mesna provided the best protective effect on prevention of acute renal failure compared with other SH reagents [24–27]. In a model of myoglobinuric acute renal failure, administration of glutathione ameliorated kidney function as expressed by a decrease in plasma creatinine, while depletion of glutathione worsened kidney function [24]. N-acetylcysteine (NAC), another SH reagent has been used to prevent renal failure in cases of acetaminophen overdose. Prescott et al. reported that NAC, when administered up to 10 h after acetaminophen ingestion [25], prevented renal damage. In a different model of renal ischaemia followed by 24 h of reperfusion, NAC restored kidney function to only 50% of sham-operated rats [26]. In contrast to our results, an ischaemia–reperfused heart model by Cargnoni could not show an improvement with mesna [27]. Their study was carried out in an isolated perfused heart with an added mesna concentration of 10^{-9} mol/l, while our interpretation is that the effective concentration is even lower due to its auto-oxidation [13,14]. The concentration we used was a thousand-fold higher compared to that of Cargnoni, and it was administered as a bolus just before ROS generation, resulting in an effective high concentration at the onset of reperfusion. The dose of mesna used in this study was determined after preliminary experiments with 120 and 60 mg/kg, both of which caused significant amelioration in GFR, with a less beneficial effect on FENa (data not shown). Mesna is not toxic to rats at the dose tested in this study (180 mg/kg) [28]. In fact, the LD_{50} values of intravenous mesna in rats lies between 1200 and 2000 mg/kg [28], which is much higher than the upper limit used in humans (60–70 mg/kg).

In the light of this limited data, it seems that mesna may be a better renoprotector than other SH reagents, probably since it is not just concentrated in the kidney [17], but is also reduced back to the active SH product in the tubular epithelial cells.

Assuming that the major generation of ROS occurs at the onset of reperfusion, and taking into consideration that mesna has a very short half-life in plasma, 0.36 h [17], it is clear why the greatest effect of mesna was achieved when administered just before the onset.
of reperfusion. However, significant although lesser beneficial effects on renal function, were obtained at all other times. This suggests that the concentration of mesna in reperfused tissue, plays an important role in renal protection.

Our morphological evidence, obtained by both light and electron microscopy, suggests that the beneficial effect of mesna on GFR and FE\textsubscript{Na} may be better explained as prevention of reperfusion damage rather than restoration of kidney function by mesna. Ischaemia itself caused very mild tissue damage, as seen from the histological sections. Ischaemia–reperfusion kidneys treated with mesna 5 min before the onset of reperfusion were morphologically similar to the histology of kidneys at the end of ischaemia, suggesting that mesna prevented further damage from occurring at the onset of reperfusion.

This study implies that mesna might be useful in preventing renal damage in ischaemic acute renal failure such as ischaemic acute tubular necrosis. Further investigation is needed for determination of the effectiveness of mesna in longer post-ischaemic periods. Mesna may be considered for preventive use in clinical situations such as major vascular surgery or organ transplantation, where ischaemia–reperfusion is encountered for renal dysfunction and may endanger organ viability and recovery.

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