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EBSELEN

ANTIOXIDANT CAPACITY IN RENAL PRESERVATION

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Abstract—Ebselen (PZ51) was tested for its ability to inhibit oxidative membrane damage and improve outcome of rabbit kidneys rendered cold ischaemic for 72 hr. In view of the rapid metabolism of ebselen, the antioxidant capacities of its two principal metabolites were first compared with that of the parent drug in an *in vitro* hepatic microsomal lipid peroxidation system initiated by NADPH/Fe^{3+}-ADP. The potent antioxidant activity of ebeselen was confirmed but metabolite I (2-glucuronylselenobenzanilide) exhibited no antioxidant potential up to a concentration of 50 μM; metabolite II (4-hydroxy-2-methylselenobenzanilide) did inhibit lipid peroxidation but was about 80 times less effective than the parent compound. The storage of rabbit kidneys in hypertonic citrate solution at 0° for 72 hr of cold ischaemia resulted in greatly increased susceptibility to oxidative membrane damage in both the cortex and medulla as determined by the subsequent *in vitro* formation of two markers of lipid peroxidation (Schiff's bases and thiobarbituric acid-reactive material). Inclusion of ebselen (50 μM) in the flush and storage solution led to a highly significant reduction in these oxidative markers in both regions of the kidney. Intracellular and interstitial oedema was noted in organs subjected to 72 hr cold ischaemia and was reduced by ebselen (50 μM in the flush/storage solution). The rate of post-ischaemic lipid peroxidation was found to correlate well with the extent of oedema in the renal medulla ($r = 0.84$, $P < 0.001$) but no such correlation was found in the cortex. Administration of ebselen (5.5 mg/kg i.v. and 100 μM in the flush/storage solution) did not improve the long-term survival of rabbits following autotransplantation of a single kidney stored for 48 or 72 hr. No protective effect of ebselen could be demonstrated either in terms of graded physiological function or histological outcome.

Free radical-mediated injury has been implicated as an important mechanism leading to post-ischaemic reperfusion damage in a wide variety of organs including the kidney [1]. Reoxygenation of rabbit kidneys following periods of ischaemia, both at normothermia and after longer periods of hypothermic storage, leads to highly elevated levels of lipid peroxidation [2]. This increase in oxidative stress encountered upon reperfusion is likely to be the result of a number of adverse biochemical processes occurring both during the ischaemic period itself and during the reoxygenation phase. These include elevated superoxide radical production due to increased leakiness of the mitochondrial electron transfer chain and the activation of xanthine oxidase [3]. Subsequent conversion of superoxide anions into more damaging species such as the hydroxyl radical may be stimulated upon reoxygenation due to the release of intracellular catalytic iron complexes from ferritin [4].

The observation that the degree of oxidative membrane damage in rabbit kidneys measured *in vitro* correlates well with the extent of ischaemic stress and the subsequent dysfunction of the organs upon transplantation *in vitro* [5] suggests strongly the use of antioxidants as ameliorative agents in renal reperfusion injury. Indeed, administration of a number of antioxidants and the iron chelator desferrioxamine has been shown to afford a significant degree of protection in the rabbit cold-stored kidney model [5].

Ebselen (PZ51) is a seleno-organic compound with both antioxidant and anti-inflammatory properties. Its potent antioxidant properties have been well characterized in a variety of *in vitro* systems, particularly the peroxidizing rat liver microsome model [6]. Moreover, ebselen displays a glutathione peroxidase-like activity at physiological concentrations of glutathione [7]; it has been shown to inhibit 5-lipoxygenase in rat peritoneal neutrophils and human platelets [8, 9]; cyclo-oxygenase in human platelets [9]; NADPH cytochrome P450 reductase in rat and mouse liver microsomes [10, 11]; and other flavin-based components of the electron transport chain (e.g. NADH-cytochrome b_5 reductase) [11].

Previously reported beneficial effects of ebselen *in vivo* include protection against galactosamine/endotoxin-induced hepatitis [12] and endotoxin-induced fetal resorption [13] in mice, and experimentally induced lung injury [14] and cerebral ischaemia in rats [15]. In the present study a rabbit kidney model of ischaemia and reperfusion has been employed to investigate the possible use of ebselen for improving renal function following 72 hr cold storage in hypertonic citrate solution (HCA†), a

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† Abbreviations: HCA, hypertonic citrate solution; TBA, thiobarbituric acid; MDA, malondialdehyde; LT, leukotriene.

formulation [16] which is commonly used in human organ transplant practice.

Ebselen is metabolized very rapidly *in vivo* and two principle products (metabolites I: 2-glucuronylselenobenzanilide and II: 4-hydroxy-2-methylselenobenzanilide) have been identified [17]. The speed of transformation is such that the parent drug is undetectable in human, pig or rat plasma following oral dosing [17]. Our preliminary HPLC studies (unpublished data) following both oral and intraperitoneal dosing corroborate this finding in the case of the NZW rabbit. Thus, the antioxidant efficacy of the two principle metabolites assumes immediate significance for the clinical utility of this agent. The antioxidant activity of a range of related selenoorganic compounds has been tested on iron/ADP/ascorbate-induced lipid peroxidation in rat liver microsomes [18]: the two principal metabolites were found to be ineffective in this system. We have used the iron/ADP/NADPH system since we are concerned not with the ability of ebselen and its metabolites to inhibit lipid peroxidation *per se*, but rather with the broader antioxidant capacities of the family, and in the projected context of renal preservation. Thus this study addresses the possibility that the ebselen metabolites inhibit NADPH cytochrome P450 reductase *in vitro*.

Preliminary studies in this laboratory involving intraperitoneal dosing of the rabbit showed that while ebselen had a significant protective effect on the renal cortex against lipid peroxidation after 72 hr cold ischaemia, both uptake of the drug and its metabolism were erratic (Ambrose, unpublished observation). Therefore, in the present study ebselen was administered intravenously in 3% (w/v) cremophor/saline solution both before nephrectomy and before subsequent auto-transplantation following ischaemic insult, and was also added directly to the flush/storage solution. Its effects were evaluated in terms of its ability to inhibit lipid peroxidation in the medulla and cortex measured *in vitro*, by histological examination, and by functional assessment of autografted single, stored kidneys by measurement of serum creatinine and urea levels and the monitoring of the long-term survival of the animals.

MATERIALS AND METHODS

All chemicals were Analar Grade, except chloroform and methanol which were Spectral Grade from BDH (Poole, U.K.). Ebselen (PZ51) and its metabolites I (2-glucuronylselenobenzanilide) and II (4-hydroxy-2-methylselenobenzanilide) were a kind gift from A. Nattermann and Co. GmbH (Cologne, F.R.G.).

Rat liver microsome preparation. Male Sprague–Dawley rats (wt. 200–250 g) were killed by cervical dislocation and the livers perfused via the portal vein with 50–80 mL ice-cold 0.9% NaCl, blotted dry and chopped. A 25% (w/v) suspension was made in Tris–KCl–EDTA buffer (50–125–1 mM, pH 7.4), and the samples were homogenized in 10 strokes using a Potter-Elvehjem type homogenizer. Homogenates were centrifuged at 20,000 *g* for 20 min, the supernatant collected and subjected to further centrifugation at 100,000 *g* for 60 min. The microsomal pellet was washed with 2–3 mL Tris–KCl buffer (50–125 mM), resuspended to the original volume in Tris–KCl and centrifuged at 100,000 *g* for 60 min. The pellet was again washed and resuspended in Tris–KCl to a final concentration equivalent to 1.5 g liver/mL buffer, and kept frozen under liquid nitrogen until use.

Microsomal lipid peroxidation. Peroxidation of rat liver microsomes (final microsomal protein concentration 0.5 mg/mL) was stimulated with NADPH/$Fe_2(SO_4)_3$-ADP (final concentrations 1 mM, 50 μM and 4 mM respectively). Ferric sulphate and ADP were premixed 30 min prior to initiation of peroxidation. Ebselen (in 10 μL dimethyl sulphoxide, final concentrations of 1.6, 3.2 and 10 μM) was added to the microsomal suspension 5 min prior to initiation of peroxidation with NADPH/$Fe_2(SO_4)_3$-ADP, and samples were incubated at 37° in a shaking water bath. The effect of metabolites I and II was also studied (final concentrations of 3.2, 10 and 50 μM). Aliquots (0.2 mL) of the incubation mixture were taken prior to initiation and at various time points up to 60 min. Lipid peroxidation was determined by the measurement of thiobarbituric acid (TBA)-reactive material as described previously [19].

Surgical procedure. Adult male NZW rabbits (average weight 3 kg) were anaesthetized by an i.m. injection of fentanyl fluanisone (Hypnorm; 0.2 mL/kg) followed by slow injection of diazepam (1.0 mg/kg), heparin (300 U/kg) and frusemide (0.3 mg/kg). Oxygen (2 L/min) was supplied via an open face mask. The right kidney was exposed through a midline abdominal incision and removed after careful dissection and ligation of the vessels and ureter. The warm ischaemic period was less than 2 min.

The renal artery was cannulated and flushed with 40 mL cold HCA or HCA containing ebselen (100 μM in 0.2% ethanol (v/v), final concentration) from an ice-cooled bag suspended at a height of 1.5 m. The kidney was placed in a sterile beaker containing ice-cold flush solution and surrounded by ice within a closed polystyrene container held in a refrigerator. The storage temperature was thus maintained at a steady 0° for 72 hr.

Determination of lipid peroxidation in rabbit kidneys. Nephrectomies were performed as described above. Kidneys were either flushed with 40 mL HCA or HCA containing ebselen (50 μM in 0.1% ethanol (v/v), final concentration), and stored in the same solution for 72 hr at 0°.

After the storage period, kidneys were dissected into cortex and medulla, suspended (5% w/v) in phosphate-buffered saline (40 mM KH_2PO_4: K_2HPO_4; pH 7.4) and homogenized using a Potter–Elvehjem homogenizer. The protein content of the homogenates was determined by the method of Lowry *et al.* [20] using bovine serum albumin as standard.

The susceptibility of kidney homogenates to lipid peroxidation was determined by incubating homogenates at 37° in open vessels with mechanical shaking for 60 min. Two markers of lipid peroxidation were determined. To measure the formation of lipid-soluble Schiff's bases, duplicate aliquots (1 mL) were

removed at 0 and 60 min of incubation, the lipids extracted into chloroform: methanol (2:1; 4 mL) and monitored for a fluorescence maximum between 400 and 450 nm when excited at 360 nm on a Perkin–Elmer LS5 spectrofluorimeter. Aliquots (2 mL) were also removed in duplicate at 0 and 60 min of incubation and the formation of TBA-reactive material measured by the method described by Suematsu and Abe [19], monitoring the fluorescence at an excitation of 515 nm and an emission of 553 nm. A standard curve was constructed using a series of different concentrations of malondialdehyde (MDA) tetraethylacetal and results were expressed as nmol MDA equivalents/mg protein/hr. Statistical analysis was performed using Student's *t*-test.

Effect of ebselen on kidney viability. Ebselen (1 mM) was dissolved in isotonic saline containing 3% cremophor (w/v) and 60 mL of ebselen solution or vehicle only was administered i.v. to rabbits (dose 5.5 mg/kg) 10 min prior to nephrectomy. Kidneys were stored at 0° for 48 or 72 hr in HCA containing ebselen (100 μM) as described previously.

After the storage period, kidneys were autografted to assess the effect of ebselen on renal preservation. The donor rabbit was anaesthetized and opened along the same mid-line incision. The left kidney was carefully dissected out and removed after clamping the renal vessels. The stored right kidney was then autografted into the left renal bursa and the renal vessels and ureter were anastomosed by standard microsurgical techniques. Ebselen solution (60 mL; 1 mM) was administered i.v. with a Braun Perfusor VI pressure pump at a rate of 120 mL/hr starting 15 min prior to reperfusion, and the rabbits were allowed to recover.

Rabbits developing uremia were killed and autopsied as soon as clinical symptoms were observed. Renal function was assessed by: observation of kidney colour and urine production immediately after revascularization; daily measurment of blood urea (urease method, Boehringer Mannheim, Lewes, U.K.) and serum creatinine (alkaline picrate method, Boehringer Mannheim) for 5 days and every 3 days thereafter; the clinical appearance of each animal; evidence of urine production; and by the macroscopic and microscopic appearance of each kidney at autopsy.

Histological examination. Lateral slices were taken from kidneys prior to homogenization and analysis of products of lipid peroxidation, and stained by hematoxylin and eosin and periodic acid-Schiff techniques. Ischaemic damage was categorized and oedema (interstitial and intracellular) was scored blind on a scale of 0 (absent)–4 (severe).

Kidneys from rabbits exhibiting symptoms of chronic renal failure were harvested and preserved in formal saline prior to histological examination.

RESULTS

NADPH/Fe^{3+}-ADP was found to be a potent stimulator of microsomal lipid peroxidation (Fig. 1a). The reaction was essentially complete at 30 min and maximal microsomal lipid peroxidation was found to be between 40 and 55 nmol MDA equivalents/mg protein.

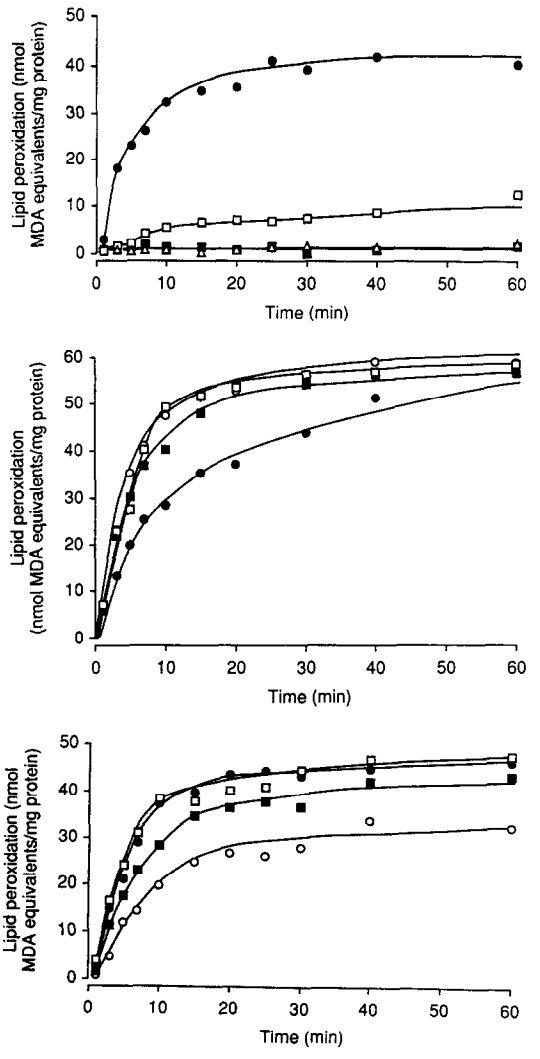

Fig. 1. The effect of [ebselen] (a), [metabolite I, 2-glucuronylselenobenzanilide] (b) and [metabolite II, 4-hydroxy-2-methylselenobenzanilide] (c) on hepatic microsomal lipid peroxidation initiated by NADPH/$Fe_2(SO_4)_3$-ADP. (●) control; (○) 50 μM; (■) 10 μM; (△) 3.2 μM (□) 1.6 μM. Values represent means of three determinations.

Ebselen was found to be a very effective inhibitor of lipid peroxidation in this model system. At a concentration of 1.6 μM, peroxidation was very significantly decreased, and was completely inhibited by 3.2 μM and 10 μM ebselen over the course of 1 hr (Fig. 1a). These results agree well with previously published studies [6, 7].

The effects of ebselen metabolites I and II towards lipid peroxidation are shown in Fig. 1b and c respectively. Metabolite I displayed no antioxidant activity and indeed was found to be slightly pro-oxidative up to a concentration of 50 μM. Metabolite II however did inhibit lipid peroxidation but was much less effective than ebselen itself. At 1.6 μM, no effect on the rate of lipid peroxidation was

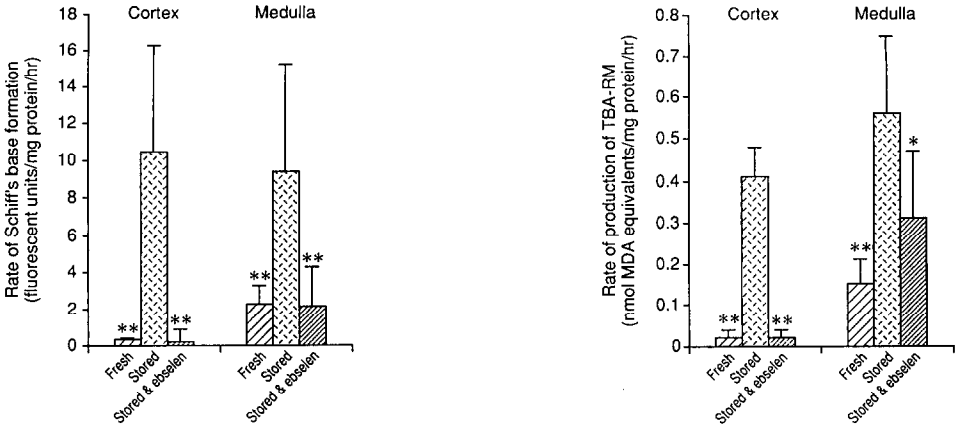

Fig. 2. The effect of ebselen on lipid peroxidation (a, Schiff's base; b, TBA-reactive material) in homogenates prepared from the cortex and medulla of kidneys stored for 72 hr at 0° in HCA ± ebselen (50 μM). **P < 0.005; *P < 0.05 compared with untreated stored organs. Values represent means and SD of six separate determinations performed in duplicate.

observed; 10 μM metabolite II displayed a faint antioxidant effect; and 50 μM inhibited peroxidation by approximately 40% over 1 hr.

The storage of rabbit kidneys at 0° in hypertonic citrate solution for 72 hr of cold ischaemia resulted in dramatic ($P < 0.005$) increases in oxidative membrane damage in both the cortex and medulla as determined by the subsequent *in vitro* formation of two markers of lipid peroxidation, i.e. Schiff's bases (Fig. 2a) and TBA-reactive material (Fig. 2b). Inclusion of ebselen (50 μM), in the HCA flush and storage solution very significantly ($P < 0.005$) reduced the formation of both markers of lipid peroxidation in the cortex of kidneys following storage for 72 hr when compared to untreated controls stored for the same length of time (Fig. 2a and b). Indeed, lipid peroxidation in the cortex of kidneys rendered cold ischaemic in the presence of ebselen was reduced to the low levels found in fresh organs. Ebselen was also highly effective ($P < 0.005$) compared with stored controls) at inhibiting Schiff's base formation in the medulla of kidneys following cold ischaemia, again the values being reduced to fresh levels (Fig. 2a). The production of TBA-reactive material in the medulla was significantly ($P < 0.05$) reduced by ebselen inclusion in the flush and storage solutions to about 50% of the value found in stored, untreated organs (Fig. 2b).

Intracellular and interstitial oedema was noted in the medulla of all stored kidneys, and was present to a lesser degree in the cortex. Oedema was moderate at worst. Congestion, haemorrhage and necrosis were not present.

Oedema was scored on a scale of 0 (absent)–4 (severe). Ebselen reduced medullary oedema from 2 to 1.33, and cortical oedema from 1 to 0.33. These reductions failed to reach statistical significance (N = 6 in each group). The extent of oedema in the medulla correlated well with the rate of lipid peroxidation as determined by the production of TBA-reactive material ($r = 0.84$, $P = 0.0007$) and inversely with ebselen treatment (Fig. 3a). However, in the cortex there was no such correlation between lipid peroxidation measured *in vitro* and oedematous change (Fig. 3b; $r = 0.48$, $P = 0.12$).

All rabbits (N = 6 for control group and ebselen-treated group) which were autografted with kidneys subjected to 72 hr cold ischaemia developed uremia by day 4, and no rabbits survived beyond 8 days. After 48 hr storage, 50% (3/6) of the rabbits in the untreated control group survived indefinitely, but only 17% (1/6) of the ebselen-treated rabbits survived beyond 10 days. However, there was no statistically significant difference between these two groups: thus no beneficial or deleterious effect of ebselen was demonstrated. Nor was it possible to show a beneficial or deleterious effect of ebselen either in terms of graded physiological function (plasma urea and creatinine levels) or in the subsequent histological appearance of the kidneys. In cases where renal function was insufficient to support life, either complete infarction had occurred (Fig. 4a) or there was gradual severe necrosis of the whole kidney (Fig. 4b). That necrosis was gradual in these instances was illustrated by the demarcation of successive necrotic zones by acute inflammatory infiltrate and casts (necrotic debris); large calcific deposits were also prominent.

DISCUSSION

In this study the rabbit kidney model was employed to investigate the possible use of ebselen for improving renal function following damaging periods of cold ischaemia and reperfusion.

An important factor in the therapeutic use of ebselent appears to be the speed of its metabolism. Following oral administration, untransformed ebselen is not detectable in plasma but is rapidly transformed into a variety of metabolites of which two [metabolites I (2-glucuronylselenobenzanilide) and II (4-hydroxy-2-methylselenobenzanilide)] are

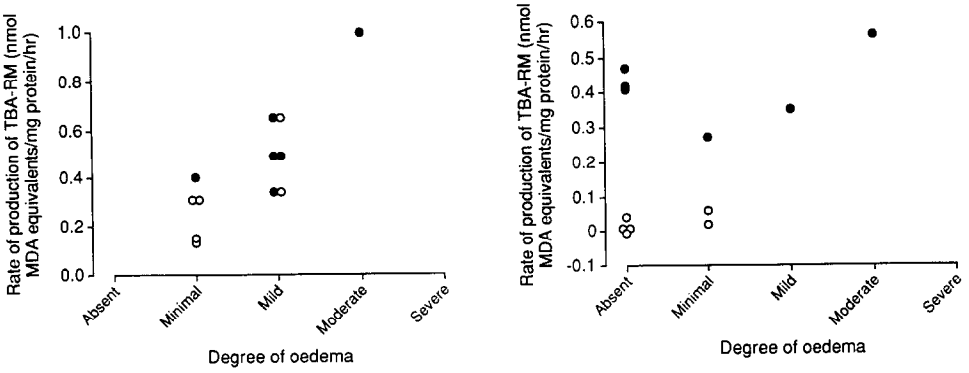

Fig. 3. The correlation between the rate of lipid peroxidation (TBA-reactive material) and the extent of oedema in the cortex (a) and medulla (b) of kidneys stored for 72 hr at 0° in HCA + ebselen (50 μM) (○) or − ebselen (●).

prominent [17, 21, 22]. Thus, if the antioxidant activity of ebselen plays an important part in its pharmacological effects, then the antioxidant efficacy of the principal metabolites are an important consideration in the clinical utility of this agent.

The *in vitro* microsomal studies reported here demonstrate that the antioxidant efficacy of the two principal metabolites towards lipid peroxidation stimulated by iron/ADP/NADPH is much lower than the untransformed drug and probably too low for any antioxidant effect to occur *in vivo*. However, the powerful inhibitory effect of ebselen itself toward lipid peroxidation shown in previous studies [6, 7] was confirmed.

This study demonstrates that exposure of rabbit kidneys to clinically relevant periods of cold ischaemia in a storage medium commonly used for human organ transplants results in very highly elevated rates of lipid peroxidation measured *in vitro* in both the cortex and medulla. These results are in good agreement with our previous findings [1, 2], which demonstrated a good correlation between the susceptibility of rabbit kidneys to lipid peroxidation and their subsequent physiological dysfunction. If, indeed, free radical-mediated events such as lipid peroxidation are important factors in reperfusion damage, then it follows that for the antioxidant activity of ebselen to be of clinical relevance in renal preservation, a system by which untransformed ebselen is presented directly to the kidney is necessary. The low solubility of ebselen in aqueous solution requires the use of such solubilizing agents as ethanol (for the micromolar range) and cremophor EL (for the millimolar range). Investigations of the renal toxicity of these treatment regimes showed no significant histological or ultrastructural damage.

The direct flushing of the kidney with HCA containing 50 μM ebselen led to a highly significant ($P < 0.005$) protective effect of renal cortex and medulla from lipid peroxidation following 72 hr cold ischaemia. Except for the production of TBA-reactive material in the medulla, which was reduced by ebselen by about 50%, all other markers of lipid peroxidation were reduced to the very low levels found in fresh organs. In addition to the peroxidation of fatty acyl chains with three or more double bonds [23], TBA-reactive substances are produced as side products during prostaglandin and thromboxane synthesis [24]. Previous experiments in this laboratory have demonstrated that approximately 50% of the TBA-reactive material produced in the post-ischaemic medulla is formed by this mechanism [25]. The almost complete inhibition of Schiff's base formation in the medulla in comparison with the only partial reduction in TBA-reactive material by ebselen is consistent with the effect of ebselen as a powerful antioxidant but poor inhibitor of cyclooxygenase in this model. In contrast, ebselen has been reported to inhibit cyclooxygenase in human platelets with an IC_{50} of around 5 μM [9].

The kidney, particularly the medulla [26], is capable of synthesizing a range of prostaglandins including thromboxane and $PGF_{2\alpha}$ (vasoconstrictors), prostacyclin (a vasodilator) and PGE_2. Hypoxia has been shown to stimulate the production of some of these metabolites in renal mesangial cells [27] and in whole kidneys subjected to ischaemia an imbalance in eicosanoids in favour of vasoconstriction on reperfusion has been demonstrated [28]. In addition, there is evidence to suggest that prostaglandins are involved in the development of oedema following cerebral ischaemia [29]. Thus, adverse effects on the metabolism of arachidonic acid via cyclooxygenase as a result of hypothermic storage, and the apparent inability of ebselen to inhibit this enzyme under the conditions employed, may have been partly responsible for the observed failure of renal function following the autograft of a single, stored kidney.

Lipoxygenation is another enzyme-catalysed pathway for the generation of specific lipid peroxides. Lipoxygenation of arachidonic acid results in the formation of leukotrienes (LTs) which are important mediators of vascular shock [30]. LTC_4 and D_4 cause potent vasoconstriction of the renal vascular beds [31] and LTB_4 is a powerful chemotactic agent [30]. Ebselen inhibits lipoxygenase and also speeds the breakdown of LTB_4 to inactive isomers [9]. Lipoxygenase is known to be present in the renal cortex [32], and it has been postulated that a

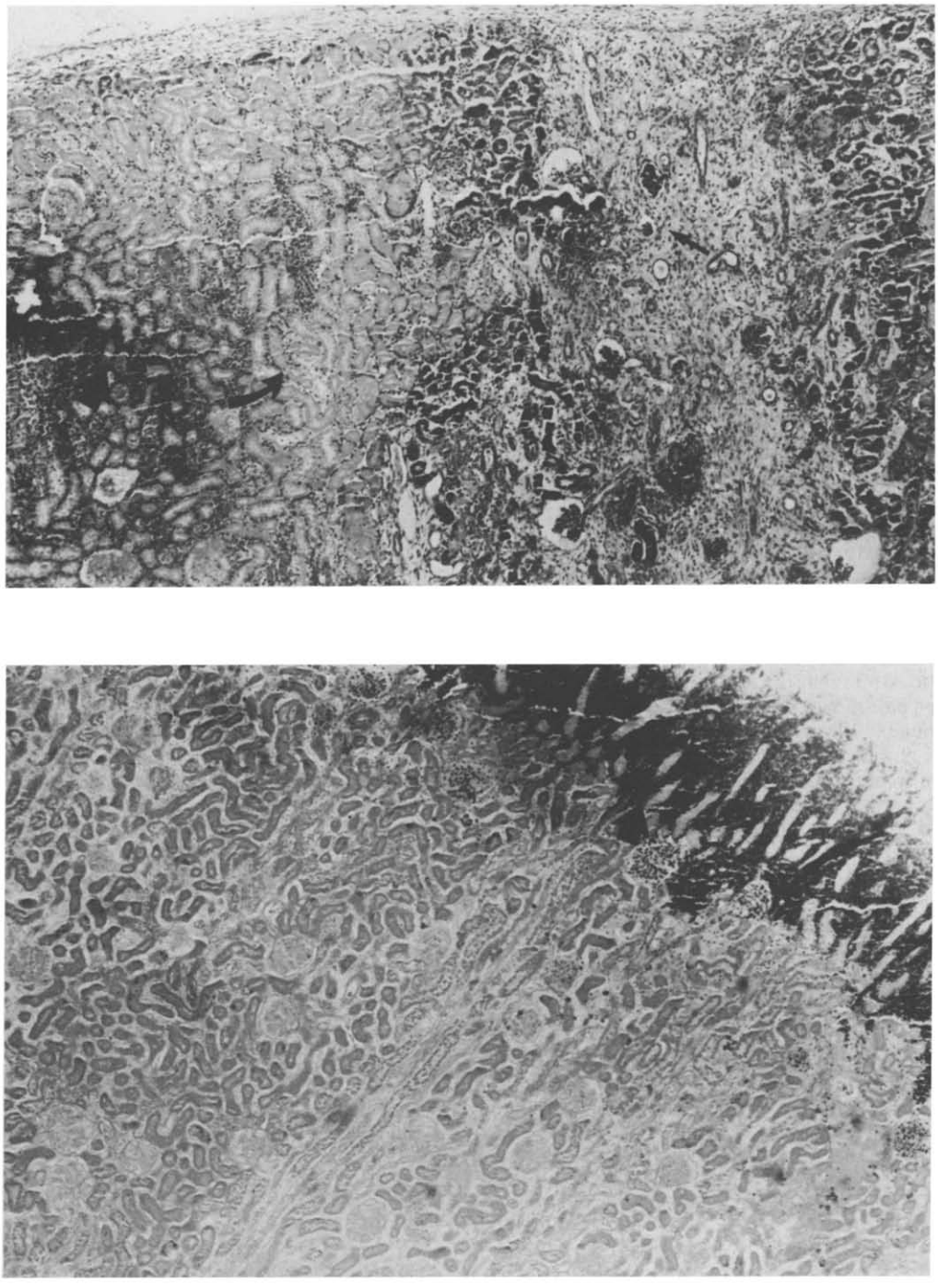

Fig. 4. Histological appearance of kidneys harvested from ebselen-treated rabbits exhibiting clinical symptoms of chronic renal failure. (a) Gradual severe cortical necrosis; (↗) necrosed tissue, (↗) fibrotic tissue, (↙) inflammatory infiltrate. (b) Cortical infarction with necrotic outlines; (↙) inflammatory infiltrate.

significant amount of lipid peroxidation occurs via this pathway in the cortex of post-ischaemic rabbit kidneys on the basis that lipid-soluble Schiff's bases were produced even in the presence of indomethacin (a cyclooxygenase inhibitor) and desferrioxamine (an iron chelator) [25]. The fact that in the present study, ebselen virtually abolished lipid peroxidation in the cortex of cold-stored kidneys suggests that its role as a lipoxygenase inhibitor may be significant under these conditions. However, it is also possible that ebselen may have been inhibiting iron-dependent or desferrioxamine-inaccessible lipid peroxidation.

Histological examination of renal slices taken from kidneys subsequently assayed for markers of lipid peroxidation demonstrated that the degree of medullary oedema correlated well with susceptibility to oxidative stress as measured by TBA reactivity, and inversely (but not significantly) with ebselen treatment. It is possible that the oedematous changes associated with ischaemia in the medulla were a consequence of damaging eicosanoid production (e.g. thromboxane A_2) during the ischaemic period itself. Although oxygen tension is low during ischaemia and intracellular conditions reductive, it is noteworthy that the content of TBA-reactive material in the medulla of stored organs is significantly higher immediately following ischaemia than in fresh organs. By contrast, cortical TBA-reactive material content does not rise significantly during the storage period, and Schiff's base reactivity does not increase in either the cortex or medulla. Furthermore, there is no correlation between the degree of oedema and these parameters of *in vitro* lipid peroxidation, suggesting that they are both free radical-mediated and post-ischaemic events. The non-significant inhibition by ebselen of medullary TBA-reactive material production implies a partial inhibition of cyclo-oxygenase under these conditions.

The failure of renal function following autograft of a single kidney stored for either 72 or 48 hr despite the intensity of the ebselen regime, both i.v. and in the flush, may have been the result of various factors. It is possible that ebselen did not reach the necessary sites of action in high enough concentrations to be effective. Certainly, significant protection against lipid peroxidation by ebselen was observed in the kidney homogenates incubated *in vitro*, which simulates the re-oxygenation of the organ *in vivo*. However, it is possible that the effectiveness of ebselen in this system was the result of access to intracellular sites only after homogenization. Although some protection by ebselen against oedematous change was observed in the intact kidney prior to homogenization, this may have been caused by low concentrations of the drug under conditions of relatively low oxidative stress.

It can be concluded that ebselen is an effective inhibitor of the increased rate of lipid peroxidation which occurs in kidneys following cold ischaemia, but that, in the present study, no definitive physiological protection against ischaemia–reperfusion damage by this agent was found. Free radical-mediated events such as lipid peroxidation are only one factor in the complex array of post-ischaemic mechanisms leading to organ deterioration. Thus the beneficial effect of ebselen in this model may only become apparent at a functional level when used in combination with other agents such as cyclooxygenase inhibitors or calcium antagonists. It is clear that further investigation is required to elucidate more fully the inter-relationships between the use of ebselen *in vivo* as an antioxidant agent in renal preservation, and the various adverse biochemical events which occur in this circumstance.

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