Urinary markers of nephrotoxicity following administration of 2-bromoethanamine hydrobromide: a comparison with hexachlorobutadiene

Ligia Delacruz, Mercè Moret, Cecilia Guastadisegni and Peter H. Bach

2-bromoethanamine hydrobromide (BEA) has been widely considered to be a target selective nephrotoxin that causes necrosis of the medulla in 24–48 h, but recent reports suggest that early cortical injury is also associated with this lesion. In order to assess the cortical effects of BEA (100 mg kg^{-1} bw single ip injection), several urinary markers of renal injury were evaluated over a 7-day period in male Wistar Albino rats. Hexachlorobutadiene (HCBD—150 mg kg^{-1} bw in peanut oil ip), a renal toxin which targets selectively for the proximal tubule, was used as a comparison. After BEA treatment, urinary levels of alanine aminopeptidase, gamma-glutamyl-transpeptidase, alkaline phosphatase and glucose increased transiently. Each of the proximal tubule marker enzymes peaked earlier following HCBD treatment and elevation of alanine aminopeptidase and gamma-glutamyl-transpeptidase was sustained for longer periods than for BEA. Following BEA treatment, lactate dehydrogenase rose prominently on day 1 followed by a return to control values on day 2 and a further rise on day 3 and remained high until the end of the study. BEA also increased the urinary excretion of total protein and albumin. After HCBD treatment, lactate dehydrogenase showed a transient elevation and glucose levels were slightly increased. Based on the present observations the changes induced by BEA administration on urinary markers of renal injury are different from those observed following HCBD treatment. These findings suggest that BEA toxicity also involves other parts of the kidney besides the papilla.

Keywords: *2-bromoethanamine hydrobromide, hexachlorobutadiene, proximal tubule injury, renal papillary necrosis, enzymuria, urinary electrolytes, proteinuria, glucosuria.*

Abbreviations: *AAP, alanine aminopeptidase; AP, alkaline phosphatase; BEA, 2-bromoethanamine hydrobromide; GGT, gamma-glutamyl-transpeptidase; HCBD, hexachlorobutadiene; LDH, lactate dehydrogenase.*

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Introduction

Renal papillary necrosis has been widely associated with analgesic abuse in humans (Gregg *et al.* 1989). In animals renal papillary necrosis has not been easily reproduced following analgesic administration (Bach and Bridges 1985) and therefore selective nephrotoxins, such as ethyleneimine (Davies and Tange 1982) and BEA, have been used to experimentally induce the lesion. BEA is a brominated alkylamine, which is readily soluble in water where it undergoes ring closure to form ethyleneimine (Dermer and Ham 1969). BEA has become a model nephrotoxin in the rat because of its high selectivity for the renal medulla. A single injection can induce a reproducibly dose-related papillary necrosis within 24–48 h (Murray *et al.* 1972). Papillary necrosis has been observed in kidney sections of rats that had been administered BEA, however proximal tubule injury was limited to hydropic changes, with no indication of cellular necrosis (Bach *et al.* 1983). Cortical degeneration has been reported at much later stages as an event secondary to the healing and fibrosis of the papilla (Cuppage and Tate 1974, Axelsen 1978). The target selectivity of BEA has been questioned as enzyme histochemical studies showed brush border loss within 8–12 h of dosing, although no overt proximal tubular necrosis was observed (Gregg *et al.* 1990). Luminal casts, which stained positively for proximal tubule marker enzymes, developed after 6 h of BEA treatment. In addition, in Nude mice BEA has also induced necrosis of the proximal tubule (Gregg and Bach 1990) and in ICR mice acute tubular necrosis has been reported as the main lesion (Wolf and Carlton 1990). These findings have suggested that BEA is less target selective than was previously assumed and it has been necessary to use clinical biochemical criteria to help establish if the proximal tubular changes are a primary or a secondary consequence. The aim of the present study was to determine the effects of a single dose of BEA on the excretion of urinary markers of renal injury in different regions of the nephron. Hexachlorobutadiene (HCBD), a nephrotoxin which targets selectively for the proximal tubule (Ishmael *et al.* 1982), was used as a comparison. The dose of HCBD (150 mg kg^{-1} bw) was selected to induce mild proximal tubule injury without gross necrosis (Lock and Ishmael 1979).

MATERIALS AND METHODS

Chemicals

2-Bromoethanamine hydrobromide was supplied by Aldrich Chemicals (Poole, Dorset) and hexachlorobutadiene (spectroscopic grade) by British Drug House Ltd (Poole, Dorset). Alkaline phosphatase, alanine aminopeptidase, gamma-glutamyl-transpeptidase, lactate dehydrogenase, protein, albumin, glucose, chloride and calcium were analysed using a Roche Cobas Bio-centrifugal autoanalyser. Reagent kits were supplied by Roche Diagnostics (Welwyn Garden, Hertfordshire). All the other chemicals were of the highest commercially available purity.

Treatment

Groups of five male Wistar Albino rats (University of Surrey strain), 5–6 weeks old and weighing 170–190 g, were given a single ip dose of a freshly prepared

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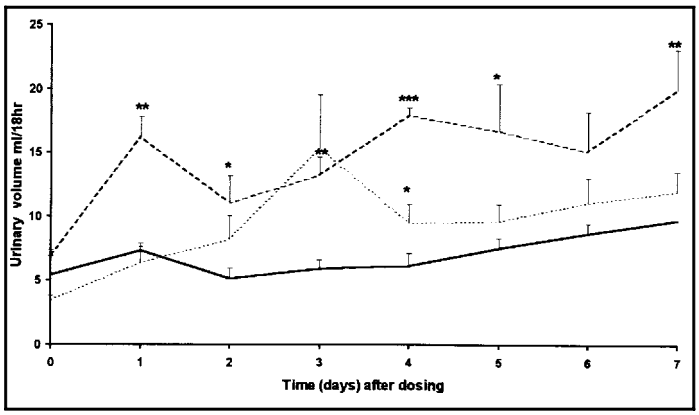

Figure 1. Changes in urinary volume in rats, before (day 0) and after a single ip dose of 100 mg BEA/kg bw (dashed line), 150 mg HCBD/kg bw (dotted line) and control group (continuous line). Each point is the mean ± SEM for $n = 5$ and those marked with asterisks differ significantly from the corresponding control value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

aqueous solution of BEA (100 mg kg^{-1} bw) or HCBD (150 mg kg^{-1} bw in peanut oil). A control group of age matched animals was also included.

Urine collection

Prior to treatment, the animals were acclimatized for 3 days. Rats were housed individually, in metal metabolic cages for 18 h per day without food. After this period they were transferred to plastic cages (Nort Kent Plastic Cages Ltd, GSI), where food (Laboratory Animal Diet No. 1 pellets, Labsure Laboratory Animal Diets, Poole, Dorset) was freely available for a 6-h period (10:00–16:00 h) each day. Tap water was supplied *ad libitum* throughout the experiment. Urine was collected at 0–5°C and the samples were centrifuged at 3000 rpm for 10 min, to separate debris and particulate matter, and analysed on the same day.

Biochemical assays

Total protein was measured by the biuret reaction (Kinsley 1939) and albumin by a modification of the bromocresol green binding assay (Doumas *et al.* 1971). Glucose was assayed by the hexokinase method (Bondar and Mead 1974) and calcium by the method of Gindler and King (1972). These parameters were expressed as mg/18 h. Chloride was measured according to the method of Zall *et al.* (1956) and results expressed as mmol/18 h. Lactate dehydrogenase (LDH), alanine aminopeptidase (AAP), gamma-glutamyl-transpeptidase (GGT) and alkaline phosphatase (AP) were assessed kinetically by standard methods (German Society for Clinical Chemistry, GSCC 1970, 1972, Mondorf *et al.* 1978) and expressed as U/18 h (1 U corresponds to a substrate conversion of 1 μmol min^{-1}).

Statistical analysis

Results are expressed as means ± SEM and were analysed by two-tail Student's t-test. $P < 0.05$ was considered significant.

Results

Urinary volume

Urinary volume rose significantly ($P < 0.001$) on the first day after BEA treatment and remained high throughout the experiment (Figure 1). By contrast, HCBD only caused a transient increase in urinary volume on days 3 and 4.

Total urinary protein and albumin

The dose of HCBD used in this study did not produce changes in total protein or albumin, as shown in Figure 2. On the contrary, animals treated with BEA showed a marked increase ($P < 0.001$) of total urinary protein (4-fold) and albumin (10-fold) on day 1. Protein excretion increased further on day 3 (10-fold, $P < 0.001$) and after day 5 both parameters returned to control levels.

Urinary enzymes

Both compounds altered the urinary excretion of brush border enzymes. BEA treatment caused a significant rise of the urinary enzyme AP from day 1 ($P < 0.001$) to day 3 ($P < 0.01$), as shown in Figure 3(A). On the other hand, HCBD significantly increased ($P < 0.05$) AP activity on days 1 and 2 (Figure 3(A)). Following BEA treatment, GGT levels were significantly increased ($P < 0.001$) only on day 2 (3-fold), returning to control values on day 3. After HCBD treatment, GGT activity rose significantly ($P < 0.001$) on day 1, to 2.5-fold of the control values and remained elevated throughout the experiment (Figure 3(B)). After BEA treatment, AAP, like GGT, was significantly increased ($P < 0.05$) on day 2 only (3-fold). On the

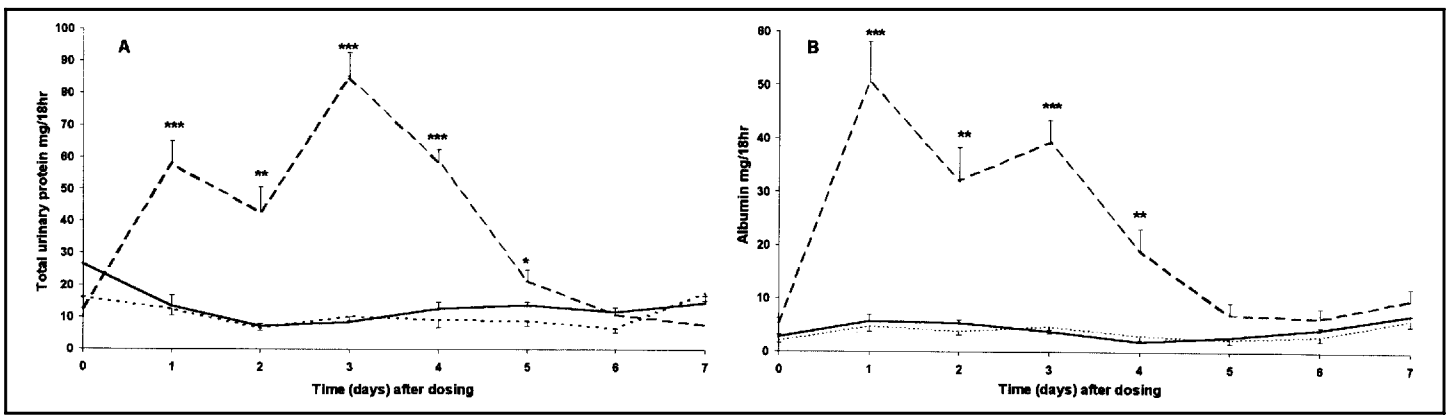

Figure 2. Excretion of total urinary protein (A) and albumin (B) in rats, before (day 0) and after a single ip dose of 100 mg BEA/kg bw (dashed line), 150 mg HCBD/kg bw (dotted line) and control group (continuous line). Each point is the mean±SEM for $n = 5$ and those marked with asterisks differ significantly from the corresponding control value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

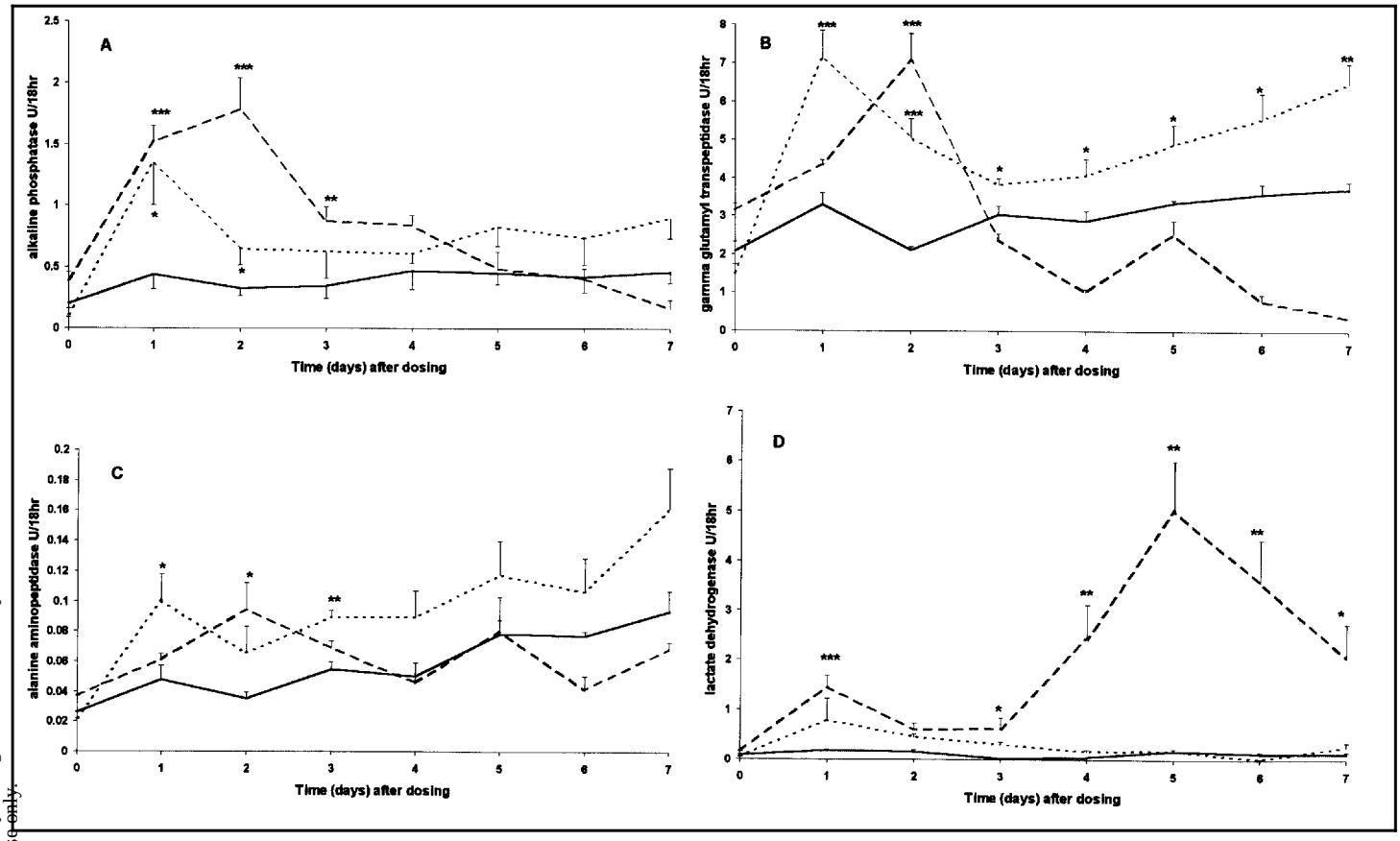

Figure 3. Excretion of urinary enzymes, alkaline phosphatase (AP) (A), gamma-glutamyl-transpeptidase (GGT) (B), alanine aminopeptidase (AAP) (C) and lactate dehydrogenase (LDH) (D) in rats, before (day 0) and after a single ip dose of 100 mg BEA/kg bw (dashed line), 150 mg HCB/kg bw (dotted line) and control group (continuous line). Each point is the mean±SEM for $n = 5$ and those marked with asterisks differ significantly from the corresponding control value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

other hand, HCBD induced a significant increase of AAP on days 1 (2.5-fold, $P < 0.05$) and 3 (2-fold, $P < 0.01$) as is shown in Figure 3(C), and the enzyme levels remained high (although not statistically significant) throughout the experiment.

BEA caused a significant ($P < 0.001$) increase in LDH excretion on day 1 (6-fold). Values returned to control levels on day 2 and started to rise again on day 3. There was a rise of at least 10-fold ($P < 0.01$) on day 5 (Figure 3(D)) and values remained high until the end of the study. Following HCBD treatment, LDH excretion showed an increase of at least 5-fold on day 1, compared with control levels. However this was not statistically significant.

Glucose and electrolytes

Urinary glucose showed a 13-fold increase ($P < 0.001$) on day 1 in the BEA-treated group and remained elevated on days 2 (3-fold, $P < 0.001$) and 3 (2-fold $P < 0.05$) and returned to control levels by day 4 (Figure 4(C)). Following HCBD treatment, urinary glucose excretion showed an increase of 2-fold, from day 1 to 3. However this was not statistically significant.

BEA-treated animals showed a 2-fold significant increase ($P < 0.01$) in urinary calcium on day 4 (Figure 4(A)). Following HCBD treatment, calcium levels were increased on day 3. Urinary chloride was transiently decreased following BEA on days 2 and 3 ($P < 0.05$) and then increased significantly ($P < 0.05$) on day 4, as shown in Figure 4(B). On the other hand, HCBD caused a 2.2-fold rise ($P < 0.05$) in urinary chloride on day 3 only.

Discussion

The results of this study show that urinary markers of renal cortical injury are affected by BEA. The brush border enzymes GGT, AP and AAP were transiently increased, demonstrating proximal tubular involvement in the lesion, which is consistent with the brush border exfoliation seen as early as 8 h after BEA administration at 100 mg kg^{-1} bw (Gregg *et al.* 1990). Moreover, in the same investigation tubular casts that stained for the brush border enzymes GGT and AP were found in the loops of Henle. These findings also highlight how a brush border enzymuria can take place in the presence of hydropic changes without cell necrosis. In other species, such as the Nude mouse, BEA at 100 mg kg^{-1} bw induced a pronounced cortical necrosis of the P_2 and P_3 segments of the proximal tubule prior to the onset of renal papillary necrosis. Luminal casts which stained positive for AP were also observed (Gregg and Bach 1990). In addition, in the Swiss ICR mouse, doses of BEA above 200 mg kg^{-1} bw mainly produced acute proximal tubular necrosis (Wolf and Carlton 1990) with a less consistent and severe papillary lesion.

The pattern of excretion of AP after HCBD administration, is consistent with the observations by Stonard *et al.* (1987) on the

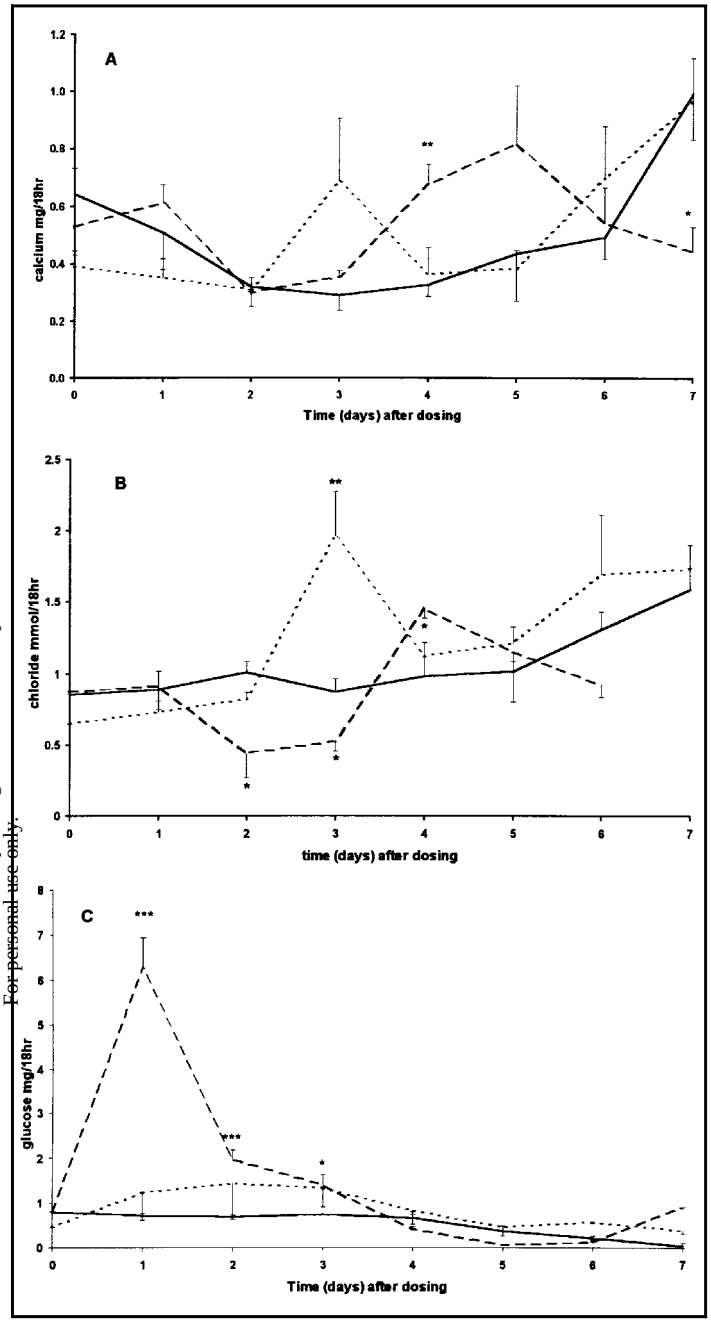

Figure 4. Excretion of urinary calcium (A), chloride (B) and glucose (C) in rats before (day 0) and after a single ip dose of 100 mg BEA/kg bw (dashed line), 150 mg HCBD/kg bw (dotted line) and control group (continuous line). Each point is the mean±SEM for $n = 5$ and those marked with asterisks differ significantly from the corresponding control value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

elevation of this enzyme within 24 h of dosing with HCBD. The early increase in GGT activity, and the fact that this was sustained throughout the study, confirm its value as a sensitive marker of proximal tubule injury.

The marked glucosuria observed in the BEA-treated animals within 24 h of dosing could have been induced by the loss of brush border at the proximal convolution which is the primary site of glucose reabsorption. The 13-fold elevation in urinary glucose observed in our study on day 1 after treatment with BEA at 100 mg kg^{-1} bw is considerably higher than the levels reported by Stonard *et al.* (1987) who observed a transient glucosuria after 200 mg kg^{-1} bw, with a maximum elevation of 3.5-fold on day 2, and by Gartland *et al.* (1988) who reported an increase of only 2-fold, 24 h after administration of a dose as high as 250 mg kg^{-1} bw. It is possible that the Wistar Albino rat strain employed in our studies, was more sensitive than Fischer 344 (Gartland *et al.* 1988) or Alderley Park (Stonard *et al.* 1987) rats.

Following BEA treatment, there was a transient increase in total protein and albumin excretion. Electrophoresis of urinary proteins (data not shown) confirmed the presence of albumin at days 1 and 2. This is consistent with data presented in Figure 2. The high levels of albumin filtered could have been due to a direct effect of BEA on the charge selectivity of the glomerular capillary wall. Perfusion of rats with cationic compounds produces fusion of the foot processes (Maddox and Brenner 1996) and scanning electron microscopy has revealed loss of the foot processes in the juxtamedullary glomeruli after BEA treatment (Sabatini *et al.* 1982). This could be the result of a reduction in the number of glomerular negative charges (Maddox and Brenner 1996). The charges of the glomerular polyanion may be reflected by the negative charges of the membrane of the red blood cells (Bernard *et al.* 1989), as determined by the binding of the cationic dye Alcian blue. Further studies with this method could help establish whether the albuminuria observed after BEA is caused by changes in glomerular perm-selectivity as a result of a reduction of the polyanion charges.

Furthermore, BEA is readily soluble in water, where it undergoes rapid cyclization to ethyleneimine (Dermer and Ham 1969), and it has been suggested that renal papillary necrosis could be mediated by ethyleneimine (Murray *et al.* 1972, Bach *et al.* 1980). However this has not been proven so far, since very little work has been published on the metabolism of BEA. It has nevertheless been suggested that because of the compound's chemical reactivity, a large range of products can be formed, including ethyleneimine and polymer species (Bach and Bridges 1985). Further studies will help elucidate which of these are the responsible species for BEA nephrotoxicity.

Following HCBD treatment there was no effect on protein excretion. By contrast, Lock and Ishmael (1979) observed a 6.5-fold increase in urinary protein within 24 h of dosing with HCBD at 100 mg kg^{-1} bw. Stonard *et al.* (1987) reported smaller increases (2-fold) in protein excretion on days 1 and 4 following a dose of HCBD as high as 200 mg kg^{-1}. The fact that our dose of 150 mg kg^{-1} did not show any effect on protein excretion could be due to differences in sensitivity by the two different strains of rats used.

Our results show that following BEA treatment, enzymuria (except LDH) and glucosuria are transient and values return to control levels, indicating a proximal tubular recovery from BEA effects. This suggests a primary effect of BEA on the proximal tubules which is different from the secondary cortical effects such as tubular atrophy (Murray *et al.* 1972) that occur late after renal papillary necrosis is well established. It is also possible that the high levels of albumin filtered could contribute to the proximal tubule injury.

LDH is a cytosolic enzyme present in proximal and distal tubules in the kidney (Piperno 1981). However, elevation of this enzyme in urine has been ascribed more specifically to distal tubular damage (Price 1982, Viau *et al.* 1984). The pattern of LDH excretion is essentially similar to the results of Bomhard *et al.* (1994) where BEA doses of 125 mg kg^{-1} bw induced a sustained elevation of the enzyme throughout their study. Our findings suggest that LDH release on day 1 followed by a return to control values on day 2 and its further rise afterwards could originate in different parts of the nephron. The observation of a sustained increase of urinary LDH in the BEA-treated group, after brush border enzymes have returned to control values, suggests a cascade of degenerative changes affecting other regions of the nephron. The release of the distal tubular antigen (RUA1), after a single ip dose of BEA at either 50 or 125 mg kg^{-1} bw (Bomhard *et al.* 1994) suggests that the papillotoxic BEA also has an effect on the distal part of the nephron. Further studies of LDH isoenzyme profiles are needed to help elucidate its proximal or distal tubular origin.

Our data show changes in calcium and chloride urinary excretion after BEA. Acute papillary necrosis induced either by BEA (Sabatini *et al.* 1981, Jaeger *et al.* 1982) or *N*-phenylanthranilic acid (Hardy and Bach 1984) has been shown to be associated with loss of calcium and a decrease in urinary chloride. However, in this study HCBD as well as BEA induced ion alterations, suggesting that these are functional changes that cannot be related to a specific type of renal injury. The sustained increase in urinary volume observed following BEA administration is consistent with previous observations on polyuria and the concentration defect associated with papillary necrosis (Fuwa and Waugh 1968, Shimamura 1975). HCBD only caused a transient increase in urinary volume on days 3 and 4 which occurred earlier than that observed by Stonard *et al.* (1987) from day 4 to day 8 following HCBD at 200 mg kg^{-1} bw. The lower dose of HCBD and the strain of rats used in our experiments could account for the different pattern of urinary volume.

In conclusion, BEA administration induced changes on urinary markers of renal injury which are different from those observed following HCBD treatment. These findings confirm that while HCBD exerts a selective effect on the proximal tubule, the toxicity of BEA appears to extend beyond the papilla to other parts of the nephron.

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References

Axelsen, R. A. (1978) Healed experimental renal papillary necrosis and cortical scarring in the rat from 2-bromoethylamine hydrobromide. *Virchows Archives of Pathology, Anatomy and Histology,* **381**, 63–77.

Bach, P. H. and Bridges, J. W. (1985) Chemically induced renal papillary necrosis and upper urothelial carcinoma. *CRC Critical Reviews in Toxicology,* **15**, 217–439.

Bach, P. H., Christian, R., Baker, J. and Bridges, J. W. (1980) The metabolism of 2-bromo-[1-^{14}C]ethan-1-amine (BEA): a model compound for inducing renal papillary necrosis (RPN). In *Mechanisms of Toxicity and Hazard Evaluation,* B. Holmsted, R. Lauwerys, M. Mercier and M. Roberfroid, eds (Elsevier, Amsterdam), pp. 533–536.

Bach, P. H., Grasso, P., Molland, E. A. and Bridges, J. W. (1983) Changes in the medullary glycosaminoglycan histochemistry and microvascular filling during the development of 2-bromoethanamine hydrobromide-induced renal papillary necrosis. *Toxicology and Applied Pharmacology,* **69**, 333–344.

Bernard, A., Amor, A. O., Roels, H. and Lauwerys, R. (1989) Red blood cell negative charges as an index of the glomerular polyanion in chronic cadmium poisoning. In *Nephrotoxicity In vitro to In vivo Animals to Man,* P.H. Bach and E.A. Lock, eds (Plenum Press, New York and London), pp. 37–41.

Bomhard, E. M., Falkenberg, F. W. and Loof, I. (1994). Changes in urinary enzymes and kidney-derived antigens after acute renal papillary necrosis in rats. *Kidney International,* **46**, Suppl. 47, S-60–S-63.

Bondar, R. J .L. and Mead, D. C. (1974) Evaluation of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenterioides* in the hexokinase method for determining glucose in serum. *Clinical Chemistry,* **20**, 586–590.

Cuppage, F. E. and Tate, A. (1974) Repair of the rat kidney following papillary necrosis produced by bromoethylamine hydrobromide. *Laboratory Investigations,* **31**, 593–601.

Davies, D. J. and Tange, J. D. (1982) Factors influencing the severity and progress of ethylenimine-induced papillary necrosis. *Journal of Pathology,* **97**, 695–703.

Dermer, O. C. and Ham, G. E. (1969) *Ethylenimine and Other Aziridines* (Academic Press, New York).

Doumas, B. T., Watson, W. A. and Biggs, H. G. (1971) Albumin standards and the measurement of serum albumin with bromocresol green. *Clinical Chemistry Acta,* **31**, 87–96.

Fuwa, M. and Waugh, D. (1968) Experimental medullary necrosis: effects of diuresis and anti-diuresis. *Archives in Pathology,* **85**, 404–409.

Gartland, K. P. R., Bonner, F. W. and Nicholson, J.K. (1988) Investigations into the biochemical effects of region-specific nephrotoxins. *Molecular Pharmacology,* **35**, 242–250.

German Society for Clinical Chemistry (1970) Standardisation of methods for the estimation of enzyme activity in biological fluids. *Zeitschrift für Klinische Chemie und Klinische Biochemie,* **8**, 658–660.

Germany Society for Clinical Chemistry (1972) Standardisation of methods for the estimation of enzyme activity in biological fluids. *Zeitschrift für Klinische Chemie und Klinische Biochemie,* **10**, 182–192.

Gindler, E. M. and King, J. D. (1972) Rapid colorimetric determination of calcium in biologic fluids with methyl thymol blue. *American Journal of Clinical Pathology,* **58**, 376–382.

Gregg, N. J. and Bach, P. H. (1990) 2-Bromoethanamine nephrotoxicity in the Nude mouse: an atypical targeting for the renal cortex. *International Journal of Experimental Pathology,* **71**(5), 659–670.

Gregg, N. J., Elseviers, M. M., De Broe, M. E. and Back, P. H. (1989) Epidemiology and mechanistic basis of analgesic associated nephropathy. *Toxicology Letters,* **46**, 141–151

Gregg, N. J., Courtauld, E. A. and Bach, P. H. (1990) Enzyme histochemical changes in an acutely induced renal papillary necrosis. *Toxicological Pathology,* **18** (1), 39–46

Hardy, T. L. and Bach, P. H. (1984) The effect of *N*-phenylanthranilic acid-induced renal papillary necrosis on urinary acidification and renal electrolyte handling. *Toxicology and Applied Pharmacology,* **75**, 265–277.

Ishmael, J., Pratt, I. and Lock, E. A. (1982) Necrosis of the pars recta (S_3 segment) of the rat kidney produced by hexachloro-1,3-butadiene. *Journal of Pathology,* **138**, 99–113.

Jaeger, P., Burckhardt, P., Wauters, J. P., Techsel, U. and Bonjour, J. P. (1982) Atteinte particulierement severe du metabolisme calcique dans la nephropathie par abus d'analgesiques. *Schweizerische Medizinische Wochenschrift,* **112**, 1791–1794.

Kinsley, G. R. (1939) The determination of serum total protein, albumin and globulin by the biuret reaction. *Journal of Biological Chemistry,* **131**, 197–200.

Lock, E. A. and Ishmael, J. (1979) The acute toxic effects of hexachloro-1:3 butadiene on the rat kidney. *Archives of Toxicology,* **43**, 47–57.

MADDOX, D. A. AND BRENNER, B. M. (1996) Glomerular ultrafiltration. In *The Kidney*, B. M. Brenner, ed. (W. B. Saunders Co., Philadelphia), pp. 286–333.

MONDORF, A. W., BREIER, J., HENDUS, J., SCHERBERICH, J. E., NAKENRODT, G., SHAH, P. M., STILLE, W. AND SCHOEPPE, W. (1978) Effect of aminoglycosides on proximal membranes of the human kidney. *European Journal of Clinical Pharmacology*, **13**, 133–142.

MURRAY, G., WYLLIE, R. G., HILL, G. S., RAMSEN, P.W. AND HEPTINSTALL, R.H. (1972) Experimental papillary necrosis I. Morphologic and functional data. *American Journal of Pathology*, **67**, 285–302.

PIPERNO, E. (1981) Detection of drug induced nephrotoxicity with urinalysis and enzymuria assessment. In *Toxicology of the Kidney*, J.B. Hook, ed. (Raven Press, New York), pp. 31–55.

PRICE, R. G. (1982) Urinary enzymes, nephrotoxicity and renal disease. *Toxicology*, **23**, 99–134.

SABATINI, S., MEHTA, P. K., HAYES, S., KURTZMAN, N. A. AND ARRUDA, J. A. L. (1981) Drug-induced papillary necrosis: electrolyte excretion and nephron heterogeneity. *American Journal of Physiology*, **241**, F14–F22.

SABATINI, S., VENKATESWARARAO, A., WILSON, A., CRUZ-SOTO, M., DE WHITE, A., KURTZMAN, N. A. AND ARRUDA, J. A. L. (1982) The effects of chronic papillary necrosis on acid excretion. *Pflügers Arch*, **393**, 262–268.

SHIMAMURA, T. (1975) Drug-induced renal medullary necrosis. Experimental and Molecular Pathology, **22**, 176–180.

STONARD, M. D., GORE, C. W., OLIVER, G. J. A. AND SMITH, I. K. (1987) Urinary enzymes and protein patterns as indicators of injury to different regions of the kidney. *Fundamental and Applied Toxicology*, **9**, 339–351.

VIAU, C., BERNARD, A. AND LAUWERYS, R. (1984) Distal tubular disfunction in rats chronically exposed to a white spirit solvent. *Toxicology Letters*, **21**, 49–52.

WOLF, D. C. AND CARLTON, W. W. (1990) Acute cortical tubular necrosis in the Swiss ICR mouse induced by 2-bromoethylamine hydrobromide. *Food and Chemical Toxicology*, **28**, 351–360.

ZALL, D. M., FISHER, D. AND GARNER, M. O. (1956) Photometric determinations of chlorides in water. *Analytical Chemistry*, **28**, 1665–1668.

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