EFFECTS OF RESERPINE AND L-CYSTEINE AND GLUTATHIONE DEPLETION ON 2-BROMOETHYLAMINE HYDROBROMIDE-INDUCED TUBULAR NECROSIS IN SWISS ICR MICE

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Abstract—Female Swiss ICR mice were injected ip with 100 or 300 mg 2-bromoethylamine hydrobromide (BEA)/kg body weight. Male Swiss ICR mice were subjected to water deprivation, or treated with 5% dextrose in water, dimethylsulphoxide, piperonyl butoxide, SKF-525A, sodium phenobarbital, β-naphthoflavone, probenecid, reserpine, diethyl maleate, buthionine sulphoximine or L-cysteine. Urine collected sequentially from male Swiss ICR mice given 300 mg BEA/kg body weight was analysed for glucose, protein, pH and specific gravity. Female mice were less sensitive to BEA than were male mice. Diuresis, antidiuresis, treatment with cytochrome P-450 inducers and inhibitors, and the antioxidant dimethylsulphoxide had no effect on the incidence or severity of tubular necrosis (TN) induced by BEA. Probenecid and L-cysteine decreased the severity, but they had no effect on the incidence of TN. Glutathione depletion by diethyl maleate and inhibition of glutathione synthesis by buthionine sulphoximine decreased the dose of BEA necessary to cause TN; buthionine sulphoximine was more effective than diethyl maleate. Reserpine decreased both the incidence and severity of TN. Glycosuria, aciduria and decreased urinary specific gravity occurred before morphological changes were seen under the microscope, indicating that the functional changes precede the morphological changes. These data indicate that glutathione is important in protecting against BEA-induced TN, that BEA or a metabolite is concentrated in the tubule epithelium by way of anion transport, and that vasoconstriction contributes to the development of BEA-induced TN.

INTRODUCTION

2-Bromoethylamine hydrobromide (BEA) produced renal papillary necrosis (RPN) in the rabbit (Oka, 1913), rat (Murray et al., 1972; Shimamura, 1972) and Syrian hamster (Carlton and Engelhardt, 1989). In the mouse (Wolf and Carlton, 1990) the primary lesion produced by BEA was proximal tubular necrosis (TN).

Mice injected ip with BEA developed proximal TN in a dose-dependent manner. Renal tubular degeneration was first evident by light microscopy at 2 hr post-treatment in mice given 300 mg BEA/kg body weight. At 6 hr post-treatment, the kidneys were grossly pale and had necrosis in greater than 90% of the cortical tubules (Wolf and Carlton, 1990).

The mechanism by which BEA produced renal lesions has not been determined, but tubular lesions could be related to enzyme inhibition. BEA in vitro irreversibly inhibited bovine and fungal amine oxidases (Kumagi et al., 1979; Neumann et al., 1975). 'Suicide' inhibition of these enzymes was due to the irreversible alkylation of sulphhydryl groups (Soper et al., 1985). Sabatini (1985) reported that BEA inhibited sodium and water transport and Na+K+-ATPase activity in toad and frog bladders in vitro. This enzyme inhibition may also be related to alkylation of sulphhydryl residues of the Na+K+-ATPase. Alkylation of sulphhydryl groups, such as those on glutathione, may be a mechanism by which BEA causes TN in vivo.

Reserpine, which causes renal vasodilation by catecholamine depletion, inhibited the development of BEA-induced RPN in the rat (Willie et al., 1972), as did diuresis (Fuwa and Waugh, 1968). Antidiuresis from water deprivation increased the severity of BEA-induced RPN in the rat (Fuwa and Waugh, 1968). The present studies were designed to further characterize the development of cortical TN in mice by altering tissue glutathione and cytochrome P-450 concentrations, and by affecting renal blood flow with diuresis, antidiuresis and reserpine treatments.

MATERIALS AND METHODS

Animals. In study 1, female Swiss ICR mice (Harlan Sprague–Dawley, Indianapolis, IN, USA), weighing 20 g, were housed in plastic rodent boxes containing wood shavings, and were fed a laboratory rodent chow diet (Purina, St Louis, MO, USA) and tap-water ad lib. For studies 2–10, male Swiss ICR
mice, weighing 20–25 g, were used. All mice were allowed a minimum of 3 days to acclimatize to laboratory conditions and were maintained in a 12-hr light/dark cycle.

**Study 1: response of female mice.** Female mice were treated with BEA to determine whether there was a sex difference in response or sensitivity. 31 female Swiss ICR mice were used. 16 female mice received an ip injection of 300 mg BEA/kg body weight (Sigma Chemical Co., St Louis, MO, USA) in sterile saline, at the rate of 0.1 ml solution/25 g body weight. Eight female mice were injected ip with 100 mg BEA/kg body weight (0.1 ml solution/25 g body weight). Seven control female mice received ip 0.1 ml sterile saline. The eight mice that received 100 mg BEA/kg and eight of the mice that received 300 mg BEA/kg were killed by CO2 asphyxiation at 1 day post-treatment. The remaining mice were killed by CO2 asphyxiation at 4 days post-treatment. The kidneys were collected and fixed in neutral buffered 10% formalin. After fixation, a mid-transverse section of each kidney was embedded in paraffin, and a 5 µm section was stained with haematoxylin and eosin (H and E). The sections were examined using light microscopy, and the lesions were scored (Table 1).

The lesion scores for each group were then totalled, and the mean lesion score was calculated. The lesion scores for each group were then totalled, and the mean lesion score was calculated.

**Study 2: sequential urinalysis.** Urine was collected at autopsy from 76 male mice (Table 2) that were sequentially killed at 0.5, 1, 2, 3, 6, 12 and 18 hr, and 3 and 10 days after a single ip injection with 300 mg BEA/kg body weight in sterile saline, at the rate of 0.1 ml/25 g body weight for this and all subsequent studies. Urine was also collected at autopsy from 55 control mice. The urine was analysed for pH, glucose and protein using a colorimetric stick test (Combistix, Ames Division, Miles Laboratories, Elkhart, IN, USA), and specific gravity was measured as refractive index using a refractometer (American Optical, Keene, NH, USA). Mean values from treated mice at each time period were compared with control values using Student’s t-test.

**Study 3: effects of diuresis and antidiuresis.** (A) 14 mice were provided with 5% dextrose in water as the sole source of liquid for 24 hr prior to ip injection of 10 of the mice with 300 mg BEA/kg body weight and four mice with 0.1 ml saline, and until they were killed by CO2 asphyxiation at 6 hr post-injection. 6 hr post-injection of BEA was selected because this was the earliest time when the TN was maximal (Wolf and Carlton, 1990). The refractive index of the urine was determined at autopsy as a confirmation of diuresis. The renal tissues were handled as in study 1.

(B) Water was withheld from 14 mice for 24 hr prior to ip injection of 10 of the mice with 300 mg BEA/kg body weight and four mice with 0.1 ml saline, and until all 14 mice were killed by CO2 asphyxiation at 6 hr post-injection. The refractive index of the urine determined at autopsy was used as an indicator of antidiuresis. The renal tissues were handled as in study 1.

12 mice were injected ip with 300 mg BEA/kg body weight, killed at 6 hr post-treatment and used as positive controls in studies 3–9. The number of mice that developed TN were statistically compared with the positive control mice using a chi-square contingency table test in this and in studies 4–7 and 9. TN was the only renal lesion that was seen in mice 6 hr post-treatment with BEA. In mice that did not develop TN, no other renal lesions were seen. The renal tissues were handled as in study 1.

**Study 4: effects of dimethylsulphoxide.** 13 mice were injected ip with 0.1 ml dimethylsulphoxide (DMSO; Fisher Scientific, Fair Lawn, NJ, USA). 10 of the DMSO-treated mice also received a simultaneous ip injection of 300 mg BEA/kg body weight. All mice were killed by CO2 asphyxiation 6 hr post-treatment. The renal tissues were handled as in study 1.

**Study 5: effects of cytochrome P-450 inhibition and induction.** (A) 12 mice received an ip injection of 600 mg piperonyl butoxide (Aldrich Chemical, Milwaukee, WI, USA)/kg body weight in peanut oil (0.1 ml solution/25 g body weight), 1 hr prior to ip injection with 300 mg BEA/kg body weight. Four mice and protein using a colorimetric stick test (Combistix, Ames Division, Miles Laboratories, Elkhart, IN, USA), and specific gravity was measured as refractive index using a refractometer (American Optical, Keene, NH, USA). Mean values from treated mice at each time period were compared with control values using Student’s t-test.

**Study 3: effects of diuresis and antidiuresis.** (A) 14 mice were provided with 5% dextrose in water as the sole source of liquid for 24 hr prior to ip injection of 10 of the mice with 300 mg BEA/kg body weight and four mice with 0.1 ml saline, and until they were killed by CO2 asphyxiation at 6 hr post-injection. 6 hr post-injection of BEA was selected because this was the earliest time when the TN was maximal (Wolf and Carlton, 1990). The refractive index of the urine was determined at autopsy as a confirmation of diuresis. The renal tissues were handled as in study 1.

(B) Water was withheld from 14 mice for 24 hr prior to ip injection of 10 of the mice with 300 mg BEA/kg body weight and four mice with 0.1 ml saline, and until all 14 mice were killed by CO2 asphyxiation at 6 hr post-injection. The refractive index of the urine determined at autopsy was used as an indicator of antidiuresis. The renal tissues were handled as in study 1.

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**Study 4: effects of dimethylsulphoxide.** 13 mice were injected ip with 0.1 ml dimethylsulphoxide (DMSO; Fisher Scientific, Fair Lawn, NJ, USA). 10 of the DMSO-treated mice also received a simultaneous ip injection of 300 mg BEA/kg body weight. All mice were killed by CO2 asphyxiation 6 hr post-treatment. The renal tissues were handled as in study 1.

**Study 5: effects of cytochrome P-450 inhibition and induction.** (A) 12 mice received an ip injection of 600 mg piperonyl butoxide (Aldrich Chemical, Milwaukee, WI, USA)/kg body weight in peanut oil (0.1 ml solution/25 g body weight), 1 hr prior to ip injection with 300 mg BEA/kg body weight. Four mice

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**Table 1. Lesion scoring criteria for the mouse kidney using light microscopy**

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No lesions</td>
</tr>
<tr>
<td>1</td>
<td>1–3 PCT per 20 x objective field were necrotic</td>
</tr>
<tr>
<td>2</td>
<td>No tubular casts</td>
</tr>
<tr>
<td>3</td>
<td>Approximately 10% PCT were necrotic</td>
</tr>
<tr>
<td>4</td>
<td>Rare casts</td>
</tr>
<tr>
<td>5</td>
<td>Approximately 25% PCT were necrotic</td>
</tr>
<tr>
<td></td>
<td>Casts in 50% of distal nephron</td>
</tr>
<tr>
<td></td>
<td>Approximately 50% PCT were necrotic</td>
</tr>
<tr>
<td></td>
<td>Casts filled distal nephron</td>
</tr>
<tr>
<td></td>
<td>&gt;75% of PCT were necrotic</td>
</tr>
</tbody>
</table>

**Table 2. Sequential urinalysis data from male Swiss ICR mice treated with 300 mg BEA/kg body weight**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>3 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (colorimetric units)</td>
<td>2.1</td>
<td>2.3</td>
<td>2.3</td>
<td>2.6</td>
<td>2.9*</td>
<td>2.9*</td>
<td>2.7</td>
<td>3*</td>
<td>2</td>
<td>3.5*</td>
</tr>
<tr>
<td>SD</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
<td>0.6</td>
<td>0.6</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>No. of mice</td>
<td>55</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>pH</td>
<td>6.9</td>
<td>6.8*</td>
<td>6.4*</td>
<td>6.8</td>
<td>6.8</td>
<td>7.1</td>
<td>6.8</td>
<td>6.8</td>
<td>6.3*</td>
<td>6.3*</td>
</tr>
<tr>
<td>SD</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
<td>0.6</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>No. of mice</td>
<td>54</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>No. positive for glucose</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>No. of mice</td>
<td>54</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Refractive index</td>
<td>226</td>
<td>183*</td>
<td>223</td>
<td>175*</td>
<td>208</td>
<td>205</td>
<td>195</td>
<td>136*</td>
<td>128*</td>
<td>165</td>
</tr>
<tr>
<td>SD</td>
<td>49</td>
<td>50</td>
<td>41</td>
<td>73</td>
<td>58</td>
<td>58</td>
<td>21</td>
<td>36</td>
<td>49</td>
<td>66</td>
</tr>
<tr>
<td>No. of mice</td>
<td>52</td>
<td>11</td>
<td>12</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Those values marked with asterisks differ significantly (Student’s t-test) from the corresponding control value (*P < 0.05).
BEA-induced tubular necrosis in mice

received ip piperonyl butoxide solution and 0.1 ml saline. All mice were killed 6 hr after treatment with BEA or saline. The renal tissues were handled as in study 1.

(B) 12 mice received ip 75 mg SKF-525A (Smith, Kline and Beecham, Philadelphia, PA, USA)/kg body weight in saline (0.1 ml solution/25 g body weight), 1 hr prior to ip injection with 300 mg BEA/kg body weight. Four mice received ip SKF-525A and 0.1 ml saline. All mice were killed 6 hr after treatment with BEA or saline. The kidneys were handled as in study 1.

(C) 14 mice received ip 80 mg sodium phenobarbital/kg body weight in saline (0.1 ml solution/25 g body weight), once daily for 3 days. Four mice were given ip 0.1 ml saline once daily for 3 days. 10 of the mice given phenobarbital and the four mice given only saline received an ip injection of 100 mg BEA/kg body weight on day 4. All mice were killed by CO₂ asphyxiation 6 hr after the injection of BEA. The renal tissues were handled as in study 1.

(D) 14 mice received ip 40 mg β-naphthoflavone/kg body weight in saline (0.1 ml solution/25 g body weight). 12 mice were killed by CO₂ asphyxiation 30 min after the probenecid. Four mice received ip probenecid and 0.1 ml saline. All mice were killed by CO₂ asphyxiation 6 hr after the injection of BEA. The renal tissues were handled as in study 1.

Study 6: effects of organic anion transport inhibition. 16 mice were given ip 200 mg probenecid (Merck, Sharp and Dohme, Rahway, N J, USA)/kg body weight in DMSO (0.1 ml solution/25 g body weight). 12 mice received ip 300 mg BEA/kg (0.1 ml/25 g body weight) 30 min after the probenecid. Four mice received ip probenecid and 0.1 ml saline. All mice were killed by CO₂ asphyxiation 6 hr after the probenecid and saline injection. The renal tissues were handled as in study 1.

Study 7: effects of reserpine. 14 mice received an ip injection of 3.75 mg reserpine/kg body weight (Sigma Chemical Co.) in DMSO (0.1 ml solution/25 g body weight). 10 of the treated mice were injected ip with 300 mg BEA/kg 18 hr after the injection of reserpine, and four mice with 0.1 ml saline. All mice were killed by CO₂ asphyxiation 6 hr after the injection of BEA. The renal tissues were handled as in study 1.

Study 8: effects of glutathione depletion. (A) 28 mice were injected ip with 1 ml diethyl maleate (DEM)/kg body weight (Sigma Chemical Co.), diluted with peanut oil to 200 µl/ml and given at 0.1 ml/20 g body weight. 1 hr after DEM administration, 12 mice received ip 300 mg BEA/kg, 12 received 100 mg BEA/kg and four received 0.1 ml saline. All mice were killed 6 hr after the injection of BEA. The renal tissues were handled as in study 1.

(B) 44 mice were injected ip with 600 mg buthionine sulfoximine (BSO)/kg body weight (Sigma Chemical Co.) in saline, at 0.1 ml solution/10 g body weight. 2.5 hr prior to BSO administration, 12 mice received ip 300 mg BEA/kg, 12 received 100 mg BEA/kg, eight received 50 mg BEA/kg, eight received 25 mg BEA/kg and four mice received 0.1 ml sterile saline. All mice were killed by CO₂ asphyxiation 6 hr after the injection of BEA. The renal tissues were handled as in study 1.

Study 9: effects of L-cysteine. 12 mice received ip 150 mg L-cysteine free base (Sigma Chemical Co.)/kg body weight in saline (0.1 ml solution/25 g body weight). L-cysteine was given 15 min prior to, and 30 min after, ip injection of 300 mg BEA/kg. Four mice received the L-cysteine injections and 0.1 ml saline. All mice were killed by CO₂ asphyxiation 6 hr after the injection of BEA or saline. The renal tissues were handled as in study 1.

RESULTS

Study 1

Female mice injected with 100 mg BEA/kg body weight had no gross or microscopic renal alterations. Female mice, 24 hr after injection with 300 mg BEA/kg, were less active than controls. At autopsy, the renal cortex was pale. At 24 hr in six of eight treated female mice, the proximal convoluted tubules of the superficial and mid-cortical nephrons were necrotic, and those mice that developed TN (Plate 1) had a mean TN index of 3.8. The other two female mice did not have any renal lesions. The proximal convoluted tubules of the juxtamedullary nephrons and tubules of the medullary rays were not affected. Approximately 50% of the distal tubules and collecting ducts contained hyaline casts. The renal papillary interstitium was expanded by oedema fluid. Collecting duct epithelium was attenuated, and scattered clusters of thin limbs of Henle and vasa recta were necrotic (Plate 2). Two of the six female mice with TN also had total RPN of the distal one-third of the renal papilla and intermediate RPN of the proximal two-thirds of the papilla. Cortical changes in two of the six female mice with TN were very mild and consisted of necrosis of scattered proximal convoluted tubules of the mid-cortical nephrons (Plate 3). The associated distal tubules contained hyaline casts. The interstitium of the renal papilla was expanded by oedema fluid, and scattered thin limbs of Henle and vasa recta were necrotic.

Five of eight (63%) female mice injected with 300 mg BEA/kg body weight survived to 4 days post-treatment. At autopsy, the kidneys of all five mice were pale. Microscopically, approximately 50% of the proximal convoluted tubules were lined by regenerating epithelium composed of cuboidal epithelial cells with basophilic cytoplasm and large, oval, vesicular nuclei (Plate 4). Rarely, proximal convoluted tubules contained granular eosinophilic necrotic debris and were lined by markedly attenuated epithelium. The renal papilla was shrunken, and the ducts within the inner medulla were lined by markedly attenuated epithelium. Scattered thin limbs of Henle and vasa recta were necrotic.

Study 2

The results of sequential urinalysis of male mice given 300 mg BEA/kg body weight are given in Table 2. Mean urinary protein was significantly greater (P < 0.05) than controls at 3, 6 and 18 hr and 10 days post-treatment. No glucose was found in the urine of control mice; thus, any glucose in the urine of treated mice was considered significant. Mean urinary pH
was significantly ($P < 0.05$) more acidic at 0.5 and 1 hr and 3 and 10 days post-treatment. Mean urinary specific gravity, measured as refractive index, was significantly lower than that in the urine of control mice ($P < 0.05$) at 0.5, 2 and 18 hr and 3 days post-treatment.

**Studies 3 and 4**

Diuresis, antidiuresis and DMSO (Table 3) had no effect on the incidence and severity of renal lesions in mice given 300 mg BEA/kg body weight, and no renal lesions were seen in the control mice. In these and subsequent studies, TN was the only renal lesion that was seen in mice 6 hr post-treatment with BEA. In mice that did not develop TN, no other renal lesions were seen. The mean refractive index of urine from control mice undergoing diuresis or antidiuresis was 108 and 323, respectively. These values differed significantly ($P < 0.05$) from the non-treated controls (Table 2), indicating that the desired effect on urine concentration was achieved.

**Study 5**

The incidence of TN in mice given 300 mg BEA/kg body weight and either piperonyl butoxide or SKF-525A (Table 3) was not significantly different from mice given 300 mg BEA/kg alone. The mean lesion scores (Table 3), as a measure of the number of necrotic cortical tubules, in mice treated with piperonyl butoxide or SKF-525A that developed TN, were not markedly different from mice given BEA alone. Control mice that received only piperonyl butoxide or SKF-525A did not develop any renal alterations.

Mice treated with either the cytochrome monooxygenase inducers sodium phenobarbital or $\beta$-naphthoflavone or 100 mg BEA/kg did not develop renal lesions.

**Study 6**

The incidence of TN due to BEA treatment was markedly decreased in mice treated with reserpine (Table 3). Only 40% of mice treated with reserpine and BEA, significant at $P < 0.05$, developed renal cortical TN compared with 92% of the mice given BEA alone. The mean lesion score of mice treated with reserpine and BEA that developed TN was less than that of the positive controls. Mice treated with reserpine and BEA that developed TN had approximately two-thirds the number of necrotic tubules than did the positive controls. Control mice that received only reserpine did not develop renal lesions.

**Study 7**

No renal alterations were seen in mice given DEM or BSO alone, and mice given 25, 50 or 100 mg BEA/kg body weight did not develop TN. Inhibition of glutathione synthesis by BSO or DEM (Table 4) resulted in a lower dose of BEA causing TN. Inhibition of glutathione synthesis by BSO was more effective than glutathione depletion by DEM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BEA</th>
<th>Antidiuresis</th>
<th>Diuresis</th>
<th>DMSO</th>
<th>Piperonyl butoxide</th>
<th>SKF-525A</th>
<th>Probenecid</th>
<th>Reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN/no. of mice</td>
<td>11/12</td>
<td>10/10</td>
<td>10/10</td>
<td>9/10</td>
<td>10/12</td>
<td>7/9</td>
<td>10/12</td>
<td>4/10*</td>
</tr>
<tr>
<td>Mean lesion score</td>
<td>of all mice</td>
<td>4.6</td>
<td>5.0</td>
<td>5.0</td>
<td>4.4</td>
<td>3.8</td>
<td>3.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Mean lesion score</td>
<td>of mice with TN</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>4.9</td>
<td>4.5</td>
<td>4.7</td>
<td>3.3</td>
</tr>
</tbody>
</table>

In the above groups: 300 mg BEA/kg body weight was administered; antidiuresis involved water deprivation; diuresis used 5% dextrose in water as the sole liquid source; DMSO was administered simultaneously with BEA; piperonyl butoxide and SKF-525A were given 1 hr prior to BEA; probenecid was given 30 min prior to BEA; and reserpine—was given 18 hr prior to BEA.

The value marked with an asterisk differs significantly (chi-square contingency table) from the corresponding control value (*$P < 0.05$).
untreated controls (Wolf and Carlton, 1990). The proteinuric and had a more dilute urine than mice treated with BEA were aciduric, glycosuric, glomerular filtrate (Burg, 1986). At later time periods, with the majority of the fluid component of the post-treatment. Glucose and protein are normally with 300 mg BEA/kg body weight (Wolf and Carlton, et al., 1990). Differences in the excretion and metabolism of BEA, physiological differences between male and female tubules were first seen in mice at 2 hr post-treatment resulting in less sensitivity in females. Male Swiss ICR mice treated with chloroform de- 1990; Liebelt, 1986), and this may account for the Sex differences in response to a toxicant have also been reported with chloroform-induced nephrosis. Male Swiss ICR mice treated with chloroform develop TN but females do not. This difference was apparently due to more rapid metabolism of chloro- form in male mice (Carlton and Engelhardt, 1986). Endogenous testosterone induces morphological and physiological differences between male and female mice. The mechanisms involved in absorption, distribution, metabolism and excretion of BEA are not known. However, male mice excrete more protein, have more renal cortical alkaline phosphatase and known. However, male mice excrete more protein, have more renal cortical alkaline phosphatase and acidosis resulting from the nephrosis. In the present study, hydration status had no effect on lesion development in male mice. Diuresis in rats injected with BEA resulted in fewer animals developing RPN with lower mean lesion scores (Fuwa and Waugh, 1968). Water deprivation increased the incidence and mean lesion score of RPN in rats given BEA (Fuwa and Waugh, 1968), but was not effective in the present study in altering the renal lesions in mice. This difference may be due to the different site of lesion development in the mouse and rat. In a study using [14C]BEA in rats, several unidentified metabolites, but no unmetabolized BEA, were found in the bile and urine (Bach et al., 1980). Inhibition of cytochrome P-450 either by SKF-525A or piperonyl butoxide, and induction either by sodium phenobarbital or β-naphthoflavone, had no effect on the incidence or severity of TN in male mice given BEA, in the present study. These data may indicate that either BEA is the active principle, or that a metabolite other than one from the mixed-function oxidase system is the toxic principle. Probenecid decreased the severity of the BEA- induced lesions in male mice in the present study. Probenecid selectively blocks organic anion transport across the basolateral region of the cellular membrane of the proximal convoluted tubule (Berndt, 1989; Hook and Hewitt, 1986). This blockade could prevent toxic substances from entering the tubular epithelial cells through the basolateral membrane. Protection by probenecid has been reported for such nephrotoxicants as the cephalosporins, citrinin (Berndt, 1989), hexachloro-l,3-butadiene and methyl mercury (Ban and de Ceaurriz, 1988). The toxicity of these compounds is determined by the concentration of these products within the tubular epithelium, and the nephrotoxicity is decreased by probenecid which decreases the concentration of these substances within tubular epithelial cells (Berndt, 1989). Because probenecid decreased the severity of the TN in mice given BEA, it seems likely that BEA or a metabolite was concentrated within the tubular epithelium by way of organic anion transport. Reserpine decreased the incidence and severity of renal cortical necrosis in male mice given BEA in the present study. Reserpine causes vasodilation by de- pletion of catecholamines from adrenergic neurons (Weiner, 1980). The inhibition of BEA-induced renal damage by reserpine suggests that local ischaemia may be involved in the development of the nephrosis. The ischaemia may be the result of sympathomimetic effect of BEA (Wyllie et al., 1972), or it may be due to direct vascular damage resulting in vaso- constriction and thrombosis. Reserpine treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BEA 300</th>
<th>DEM 300</th>
<th>DEM 100</th>
<th>BSO 300</th>
<th>BSO 100</th>
<th>BSO 50</th>
<th>BSO 25</th>
<th>l-cysteine 300</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN/no. of mice</td>
<td>11/12</td>
<td>12/12</td>
<td>4/12</td>
<td>12/12</td>
<td>12/12</td>
<td>7/8</td>
<td>3/8</td>
<td>9/12</td>
</tr>
<tr>
<td>Mean lesion score</td>
<td>4.6</td>
<td>4.9</td>
<td>1.3</td>
<td>5.0</td>
<td>5.0</td>
<td>4.1</td>
<td>1.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Mean lesion score of mice with TN</td>
<td>5.0</td>
<td>4.9</td>
<td>3.8</td>
<td>5.0</td>
<td>5.0</td>
<td>4.7</td>
<td>3.3</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Dem was given 1 hr prior to BEA; BSO was given 4 hr prior to BEA. L-cysteine was given 15 min prior to, and 30 min after, BEA.
resulted in a decreased incidence of RPN in rats given BEA (Wyllie et al., 1972). Adrenergic nerve fibres innervate the afferent and efferent arterioles of the glomerulus (Barajas, 1978). Stimulation of renal nerves causes vasoconstriction and decreases renal blood flow (Maher, 1981). Blocking these adrenergic nerves would allow the pre- and post-glomerular arterioles to remain maximally dilated at all times, thus maintaining perfusion of the proximal tubules which are supplied by the afferent arteriole.

DEM and BSO increased the sensitivity of renal tubules to BEA in the present study; such results have been observed with other nephrotoxicants. Depletion of glutathione in mice resulted in acute nephrotoxicity with thiabendazole at doses of less than 5% of the LD50—a surprising result considering that nephrotoxicity had not been observed previously, even when the drug was given at doses near the LD50 (Mizutani et al., 1990). The treatment of rats with DEM prior to cephradinol significantly increased the nephrotoxicity of cephradinol (Kuo and Hook, 1982). The increased susceptibility to BEA after glutathione depletion suggests that glutathione depletion was a factor in the development of cortical TN in mice given BEA.

Treatment with L-cysteine decreased the severity of BEA-induced TN in mice. L-cysteine is a substrate for the enzyme γ-glutamylcysteine synthetase. Increased amounts of L-cysteine can result in increased synthesis of glutathione (Styrr, 1988). Maintaining tissue glutathione concentration with L-cysteine may be responsible for the reduced toxicity of BEA. Renal glutathione concentrations were increased in rats treated with N-acetylcyesteine prior to BEA when compared with rats given BEA alone, and large doses of N-acetylcyesteine prevented the development of RPN in rats given BEA (Thielemann et al., 1990). This protection might also be the result of increased availability of free thiol groups. BEA irreversibly binds to thiol groups (Soper et al., 1985), and this may be one possible mechanism for its nephrotoxicity.

The above studies suggest that functional changes of the proximal tubules occur prior to morphological changes, and that several factors may be involved in BEA-induced TN. First, BEA or a metabolite appears to be concentrated in the proximal tubular cells by way of an organic anion transport system. This idea is supported by the decrease in severity of TN in probenecid- and BEA-treated mice. Secondly, ischaemia may result from vasoconstriction and/or vascular damage by BEA or a metabolite. This would explain the decrease in renal perfusion that is counteracted by treatment with reserpine prior to BEA. Thirdly, BEA or a metabolite may deplete glutathione or overwhelm the glutathione antioxidant system. The marked decrease in the dose of BEA required to cause TN in glutathione-depleted mice provides evidence to support this hypothesis. One or all of these pathways may be operational and result in tubular epithelial cell damage and TN.

REFERENCES


