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Experimental Renal Papillary Necrosis in the Rat: The Selective Vulnerability of Medullary Structures to Injury

Roy A. Axelsen

Department of Pathology, University of Queensland Medical School, Brisbane, Queensland, Australia

Summary. Acute renal papillary necrosis was produced in rats by the administration of ethyleneimine. Low doses resulted in necrosis of interstitial cells, thin limbs of the loops of Henle and vasa recta, while collecting ducts were spared (subtotal renal papillary necrosis). High doses resulted in necrosis of all elements of the papilla (total renal papillary necrosis). Although the ranges of the doses that produced these two patterns of necrosis overlapped, it is clear that there is a dose dependent selective vulnerability of renal medullary structures to injury by the toxic agent studied.

Key words: Kidney – Papillary Necrosis.

Introduction

Renal papillary necrosis (RPN) is a well-known lesion that occurs in a variety of circumstances in man and that can be produced experimentally by several methods (Axelsen, 1976a). The lesion as it is usually described consists of necrosis of all elements of part or all of the papilla. A less severe form of RPN, characterized by necrosis of structures other than collecting ducts, also occurs and is seen in man in analgesic nephropathy, where it has been named early or intermediate papillary necrosis according to its severity (Burry, 1968 and 1971). In one prospective autopsy study, intermediate papillary necrosis was found in those patients who had ingested lower cumulative doses of analgesic drugs than the patients with advanced analgesic nephropathy, characterized in part by necrosis of *all* elements of the renal papilla (Burry et al., 1966). The finding suggested the existence of a dose-dependent, selective vulnerability of renal medullary structures to injury.

The lesion characterized by necrosis of interstitial cells, loops of Henle and vasa recta but not of collecting ducts may be named subtotal RPN. Experimental examples have been described and/or illustrated in rats given phenacetin (Fordham et al., 1965), ethyleneimine (Sherwood et al., 1971), 5-aminosalicylic acid

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(Calder et al., 1972) and 2-bromoethylamine hydrobromide (Murray et al., 1972). None of these authors discussed the lesions, nor did any of them comment on their resemblance to intermediate papillary necrosis in human analgesic nephropathy. The only systematic morphological studies of subtotal RPN were carried out by Axelsen (1973 and 1976), who investigated the RPN that occurs spontaneously in jaundiced Gunn rats (Gunn, 1938 and 1944) in association with the deposition of unconjugated bilirubin in the tips of the renal papillae in these animals.

The present experiments were designed to investigate systematically the occurrence of subtotal RPN in rats after the administration of ethyleneimine. A single dose of this compound is known to produce RPN in animals.

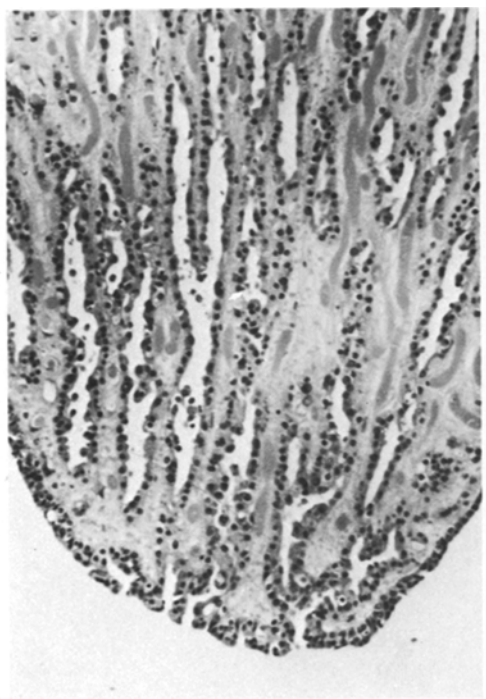
Materials and Methods

Female Sprague-Dawley rats weighing approxmately 200 g were housed up to 6 animals per cage in a non-air conditioned room and were allowed free access at all times to tap water and Purina chow. Each animal was given one subcutaneous injection of ethyleneimine in aqueous solution. Each of 8 groups of 6 or 7 rats was given one of the following dose levels of ethyleneimine: 0.25, 0.5, 1.0, 1.25, 2.0, 4.0, 6.25 and 8.0 mg/kg body weight. The concentrations of the aqueous solutions were adjusted so that the volume of each injection was 1.0 ml/kg body weight. Fifty rats were used. After 4 days, the animals were killed by cervical hyperextension and their kidneys were fixed by immersion in neutral, phosphate-buffered 4% formaldehyde. Blocks of renal tissue were cut in a plane transverse to the long renal axis and were processed by routine methods for histological examination. Paraffin sections 4 μm thick were cut parallel to the long axis of the papilla in a plane passing through or close to its tip. They were stained with haematoxylin and eosin and examined for the presence of RPN.

Results

Histological Observations. RPN was observed at a dose of 1.25 mg/kg body weight and at all higher dose levels. Two patterns of necrosis occurred. That whch was less severe (subtotal RPN) was characterized by necrosis of interstitial cells, thin limbs of the loops of Henle and vasa recta with relative sparing of collecting ducts (Fig. 1). Eosinophilic casts filled loops of Henle or vasa recta, erythrocytes and platelets filled some segments of the latter and finely granular, eosinophilic debris occupied the interstitial tissue. Collecting ducts were not normal, but had obviously suffered less damage than other structures. The size and shape of epithelial cells of the collecting ducts varied, the contours of the luminal borders of the cells were irregular, nuclear size varied more widely than in normal kidneys (Fig. 1), the cytoplasm was basophilic and necrosis of scattered, individual cells was observed. Usually, this subtotal RPN involved the distal half of the papilla.

In other animals, necrosis of all elements of the papilla (total RPN) had occurred (Fig. 2). The extent of the lesion varied. In some kidneys, only the tip of the papilla was involved while in others almost the entire papilla was necrotic. The lumina of collecting ducts were empty, and loss of epithelial cells revealed denuded basement membranes. Casts and debris from necrotic



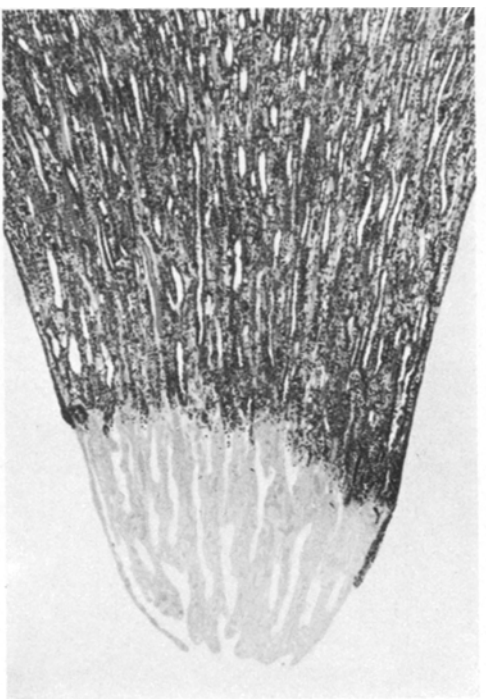


Fig. 1. Subtotal RPN. Collecting ducts are lined by epithelium. All other structures are necrotic. ×125

Fig. 2. Total RPN. All elements of the tip of the papilla are necrotic. ×50

epithelial cells filled the lumina of loops of Henle and erythrocytes and platelets were seen in vasa recta. The interstitial tissue contained granular, eosinophilic debris. The epithelium lining surviving collecting ducts above the zone of necrosis exhibited basophilic cytoplasm and mitotic figures indicative of regeneration. At the proximal margin of the necrosis an interstitial polymorphonuclear leucocytic exudate was present.

The cortex exhibited no necrosis, but dilated cortical collecting ducts were seen in some kidneys with total RPN.

In general, higher doses caused more extensive necrosis than lower doses.

The Frequencies of Subtotal and Total RPN. The numbers of animals with no lesions, subtotal RPN and total RPN at the various dose levels are shown in Figure 3. At the lowest 3 dose levels (0.25, 0.5 and 1.0 mg/kg) none of 19 rats had RPN. In the dose range 1.25–6.25 mg/kg, some animals had no lesions, others had subtotal RPN and the remainder total RPN. At the highest dose level (8.0 mg/kg) all 6 rats had total RPN. The data are presented in another manner in Fig. 4, which illustrates the fact that the range of doses within which some animals did not develop RPN is lower than that within

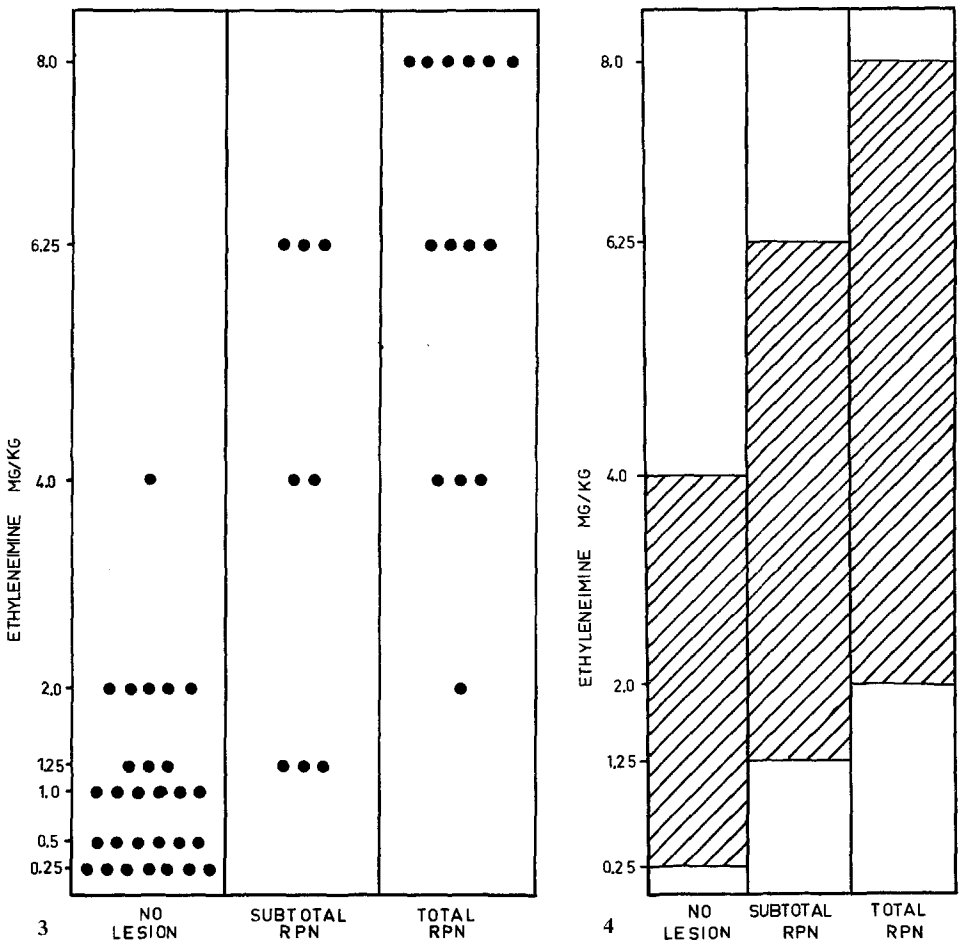


Fig. 3. Frequencies of subtotal and total RPN at the various dose levels of ethyleneimine. Each dot represents one animal

Fig. 4. The ranges of doses of ethyleneimine within which some animals exhibited no RPN, others exhibited subtotal RPN and the remainder total RPN

which others developed subtotal RPN which in turn is lower than that within which total RPN occurred. There is a considerable overlap among the three ranges, but the trend is clear.

Discussion

Total RPN is a well-known lesion in human and experimental disease. When the terms "renal papillary necrosis" or "papillary necrosis" are used, they almost always refer to total RPN. Subtotal RPN, on the other hand, is seldom mentioned in the literature. Its human counterpart, intermediate papillary necrosis in human analgesic nephropathy, is nevertheless an improtant lesion, because

its recognition allows a reasonably accurate morphological diagnosis of analgesic abuse to be made, and, in autopsy studies, augments significantly the assessed frequency of analgesic abuse based only on the recognition of advanced disease (Burry et al., 1966; Burry, 1968, 1971; Burry et al., 1974).

The present study has established that subtotal RPN can be produced at will by the exhibition of certain doses of ethyleneimine and that the occurrence of subtotal and total RPN appears to be dose dependent. Which of these two lesions would occur in a particular animal at a given dose level could nit, however, be predicted. The use of intravenous administration of this agent, rather than subcutaneous injection as was employed in the present study, could conceivably result in the appearance of a more consistent dose-response effect than that which was observed.

The finding that the occurrence of experimental subtotal RPN is dose dependent supports the validity of the observation, in a prospective autopsy study of analgesic nephropathy, that patients with intermediate papillary necrosis had consumed lower cumulative doses of analgesic drugs than those with total RPN and advanced analgesic nephropathy (Burry et al., 1966). A later autopsy study could not, however, confirm this finding (Burry et al., 1974).

Studies on renal medullary plasma flow in rats given BEA have suggested that the RPN produced by this agent cannot be attributed to ischemia (Solez et al., 1974). Paradoxically, however, the administration of reserpine was found to protect rat kidneys against the effects of BEA (Wyllie et al., 1972), an observation that remains unexplained. It is probable that ehtyleneimine, administered as a pure substance or formed in vivo after the injection of BEA (Murray et al., 1972), exerts a cytotoxic effect on the renal medulla. The occurrence of RPN is therefore probably dependent on the attainment of certain levels of concentration of ethyleneimine in the medulla. Indirect evidence which supports this hypothesis was provided by Fuwa and Waugh (1968) who found that, in rats, diuresis significantly decreased the frequency and extent of RPN produced by BEA and that antidiuresis significantly increased the extent of the lesion. Variations in the administered dose levels of ehtyleneimine and BEA should also result in variations in the concentration of ethyleneimine in the medulla and therefore in the frequency of occurrence of RPN. The findings of the present experiment support this hypothesis. At the lowest 3 dose levels of ethyleneimine (0.25, 0.5 and 1.0 mg/kg) none of 19 animals exhibited RPN, whereas all 13 animals given the highest 2 dose levels (6.25 and 8.0 mg/kg) developed either subtotal or total RPN.

An explanation for the occurrence of the selective vulnerability of medullary structures to injury, manifested in the above experiments as subtotal RPN, is less clear. It is conceivable that there exist inherently different susceptibilities of the various cellular components of the medulla to injury in the presence of a certain range of concentrations of ethyleneimine in the tissue. Any such differences would no longer be apparent if the tissue concentration of ethyleneimine reached levels lethal for all cell types. Alternatively, the movements of ethyleneimine between the various compartments of the medulla could result in the occurrence of lethal concentrations only in certain sites when a particular range of administered doses is employed. The administration of a dose outside this range could "swamp" all compartments with cytotoxic concentrations of

ethyleneimine. These alternative but not necessarily mutually exclusive proposals must remian speculative at present, as there are no data known to the author on the handling of ethyleneimine by the renal medulla.

Subtotal RPN has now been described and/or illustrated as occurring after the administration of several agents known to produce renal medullary injury: phenacetin (Fordham et al., 1965), ethyleneimine (Sherwood et al., 1971), aminosalicyclic acid (Calder et al., 1972) and BEA (Murray et al., 1972). It has been observed by the author in jaundiced Gunn rats in association with deposition of unconjugated bilirubin in the renal papillae of these animals (Axelsen, 1973; Axelsen and Burry, 1976), in Gunn rats given aspirin, phenacetin (Axelsen, 1976b) or paracetamol (Axelsen, unpublished), and in Sprague-Dawley rats given high, oral doses of aspirin and of a mixture of aspirin, phenacetin and caffeine for several weeks (Axelsen, unpublished). It appears that subtotal RPN represents a distinctive morphological expression, dose dependent in at least some circumstances, of the actions of certain toxic agents on the kidney.

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