An Ultrastructural Study of the Renal Medulla in Experimental Acute Pyelonephritis

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Received March 27, 1975

Experimental acute pyelonephritis was produced in rats by a combination of intravenous administration of Escherichia coli, strain IMRU-54, and temporary unilateral mechanical ureteral obstruction. Structural alterations of the renal medulla were studied by light and electron microscopy. Major cellular alterations occurred in the vasa recta. Tubular and interstitial cells demonstrated minimal alterations after the brief period of acute inflammation. Polymorphonuclear leukocytes within tubular lumina contained structures resembling E. coli in nonprotoplast-like form. Numerous protoplast-like organisms, to the exclusion of any other structural forms, were detected within the interstitium of the inner medulla. Nonprotoplast-like structures resembling E. coli were rarely observed in interstitium of the inner medulla. Following relief of ureteral obstruction, clearance of acute inflammation was rapid. In conclusion, hematogenous acute pyelonephritis induced by E. coli, IMRU-54, is able to inflict cytological and ultrastructural damage to structural elements of the inner and outer medulla of rats. Vasa recta incurred prominent alterations in endothelia and basement membranes, whereas tubular epithelia and interstitial cells had relatively good structural preservation. The data suggest that intravenously administered E. coli is capable to revert to a protoplast-like structure in the inner medulla.

Marked differences in susceptibility to microbial infection exist between cortex and medulla of rat and rabbit kidneys (1). Progressive bacterial infection of the renal medulla, within hours after initial colonization, underscores the importance of the medulla in the pathogenesis of acute hematogenous pyelonephritis (2). Despite the physiologically dynamic role played by the renal medulla in mammalian homeostatic regulatory mechanisms, little is known about the structural alterations of the medulla in experimental pyelonephritis by Escherichia coli, implicated as the common etiologic agent of clinically diagnosed urinary tract infections in man (3).

This communication examines the effect of E. coli infection on the ultrastructure of the rat renal medulla.

MATERIALS AND METHODS

Adult male rats (Fisher-344), weighing approximately 200 g, were used throughout the experiment. The left ureters of nine experimental rats were surgically ligated with thick Nylon thread at the ureteropelvic junction (4). Immediately following ligation and closure of the operative incision, rats in group I were injected intravenously with 1.0 ml of Ringer's solution containing approximately $3 \times 10^8$ E. coli, strain IMRU-54, obtained from the Institute of Microbiology at Rutgers University. Group II comprised nine sham-operated control rats injected with 1.0 ml Ringer's solution containing an equal number of E. coli. The left ureters of nine additional control rats (Group III) were surgically ligated and injected with 1.0 ml

This work was supported by USPHS-AM-14411-05

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Ringer's solution without *E. coli*. Group IV comprised nine control rats which were not subjected to surgical treatment and injected with 1.0 ml pure Ringer's solution. At the end of the second day after injection of *E. coli*, six rats from each of Groups I and III were relieved of their ureteral obstructions.

Three rats from each of groups I–IV were killed by exsanguination under ether anesthesia after 2, 7, and 14 days. The renal medullae of the left kidneys of all rats in each group were divided into inner and outer medullae according to the nomenclature of Peter (5). They were fixed in ice-cold, veronal-buffered 1% osmium tetroxide for 1 hr, dehydrated, and embedded in Epon-Araldite mixture No. 1 of Mollenhauer (6). From these plastic embedded tissue blocks, 1-μm-thick sections were cut and stained with alkaline toluidine blue (7) for light microscopy. Thin sections were cut by LKB Ultrotome III, doubly stained with uranyl acetate and lead citrate, and examined under a Philips 300 electron microscope. Emphasis was placed on the ultrastructural study of the inner medullae. Remaining kidney tissues were fixed in 10% neutral formalin, embedded in paraffin, sectioned, and stained with hematoxylin eosin, periodic acid Schiff's reagent, Masson's trichrome, and Brown and Brenn's modification of the Gram stain.

RESULTS

**Gross findings.** Two days postinfection with *E. coli*, kidneys of experimental rats (Group I) were hydronephrotic. On days 7 and 14, no significant hydronephrotic change was present in experimental rats whose ureteral obstructions had been relieved at day 2. Kidneys of rats from Group II, III, and IV showed no gross abnormalities at each time interval, except for rats belonging to Group III whose kidneys revealed mild temporary hydronephrotic alterations.

**Light microscopic findings.** Two days postinfection with *E. coli*, experimental rats had leukostasis in vasa recta and focal accumulation of polymorphonuclear leukocytes within the medulla. Leukocytic casts were frequently observed in lumina of collecting tubules (Fig. 1). Many leukocytes within the tubular lumina contained
Several polymorphonuclear leukocytes in the subendothelial site and within the capillary lumen. The capillary basement membrane is focally perforated (smaller arrow), and is partially denuded of lining endothelium (larger arrow). Little structural alterations present in the interstitial cells. Mag. x6700.

Phagocytized *E. coli*, best seen in sections stained with alkaline toluidine blue or Brown and Brenn's modification of the Gram stain. Structures resembling *E. coli* were less frequently observed in the interstitium. In areas where focal accumulations of leukocytes were prominent, capillary basement membranes had foci of disruption or perforation that were seen in sections stained with periodic acid Schiff's reagent. Following relief of ureteral obstruction 2 days postinfection with *E. coli*, acute inflammation diminished rapidly. In this group of experimental rats, no significant acute inflammatory processes were observed after 7 and 14 days. However, Masson's trichrome stain revealed focal interstitial fibrosis of the medulla on day 14. Comparable changes were not present in control rats.

**Electron microscopic findings.** Two days postinfection with *E. coli*, experimental rats demonstrated numerous polymorphonuclear leukocytes within the interstitium, capillaries, and tubular lumina (Fig. 2). In some instances, basement membranes of vasa recta of the inner and outer medulla were attenuated and denuded of lining endothelia, and to whose luminal surfaces polymorphonuclear leukocytes or partially degranulated platelets frequently adhered. On occasion, capillary basement
membranes were perforated. Despite the changes and damage on the capillary walls, ultrastructural alterations of tubular epithelia and interstitial cells in the inner and outer medulla were minimum. Swelling of cytoplasm and mitochondria of some of tubular and interstitial cells was present.

Phagocytized bacilli resembling *E. coli* were visible in many polymorphonuclear leukocytes within collecting tubular lumina of the experimental rats on day 2 (Fig. 3). Control rats injected with Ringer's solution did not reveal similar bacterial forms. This observation suggests that the intravenously administered *E. coli* was responsible for the observed acute pyelonephritis, and not elements of the indigenous or exogenous microflora other than the organisms used to initiate infection. *Escherichia coli* demonstrated various degrees of degradation within
membrane-bound phagolysosomal structures of polymorphonuclear leukocytes (Fig. 3). A smaller number of structures resembling *E. coli* also existed freely within the interstitium. The majority of *E. coli* in the interstitium of inner medulla existed as protoplast-like structures (Fig. 4).

On days 7 and 14, acute inflammation was difficult to detect in outer and inner medulla of experimental rats whose ureteral obstruction had been relieved on day 2. Occasionally, however, denuded areas of capillary basement membranes remained unrepaired. The inner and outer medullary interstitium demonstrated a slight increase in collagen fibers and microfilaments consistent with tropocollagen. Associated with the increase in interstitial ground substance, interstitial cells demonstrated prominence of microvesicles, coated vesicles, rough endoplasmic reticulum and, to some degree, free ribosomal particles.

Examination of control rats revealed no evidence of acute pyelonephritis or ultrastructural alterations of the medulla comparable to those noted for experimental rats.

**DISCUSSION**

A unique physiological and biochemical environment is provided by the renal medulla during the pathogenesis of pyelonephritis. Characteristics of this environment include a relatively poor circulation (2), increased levels of urea, and elevated tissue osmolarity (8). In addition, high concentration of ammonia and ammonia-
producing enzymes which inhibit complement activity (9) impair production of leukocyte chemotactic factor. Phagocytic activity of leukocytes may be reduced in the medulla due to this unique biological environment which, in addition, may also contribute to the differences in susceptibility to microbial infection between the renal cortex and medulla of rats and rabbits.

Despite the rather favorable environment provided by the medulla for bacterial colonization, the kidney of the rat is notoriously resistant to infection with *E. coli* unless preceded by mechanical ureteral obstruction as practiced in the present investigation. Following the induction of acute pyelonephritis, the most severely affected structure was the vasa recta. During the brief acute stage of inflammatory events, however, tubular epithelia and interstitial cells were relatively resistant to infection with intravenously administered *E. coli*. The rapid clearance or disappearance of acute inflammatory processes, and subsequent restoration of the structural integrity of renal medullary tissue following relief of ureteral obstruction, may be partly due to the relative resistance of tubular epithelia and interstitial cells to brief acute inflammatory events in the medulla. Diuresis, following relief of ureteral obstruction, may facilitate elimination of inflammatory cell debris and deleterious hydrolytic enzymes liberated by polymorphonuclear leukocytes. Consequently, further damage of medullary tissue by these enzymes is, for the most part, largely prevented. Whether damage of medullary tissue was due to *E. coli* or indeed due to liberation of leukocyte-derived lysosomal hydrolytic enzymes requires further study.

ACKNOWLEDGMENT

This work was supported by USPHS-AM-14411-05.

REFERENCES

1. Freedman, L. R., and Beeson, P. B. Experimental pyelonephritis. IV. Observations on infection resulting from direct inoculation of bacteria in different zones of the kidney. *Yale J. Biol. Med.* 30, 406 (1958).
2. Rocha, H., and Fekety, F. R., Jr. In "Progress in Pyelonephritis" (E. H. Kass, Ed.), pp. 211–220. F. A. Davis Co., Philadelphia, 1964.
3. Rantz, L. A. Infections of the urinary tract. *Advances in Internal Medicine* 1, 137, (1942).
4. Shimamura, T., Kissane, J. M., and Gyoerkey, F. Experimental hydronephrosis, nephron dissection, and electron microscopy of the kidney following obstruction of the ureter and in recovery from obstruction. *Lab. Invest.* 15, 629, (1966).
5. Peter, K. "Untersuchungen ueber Bau und Entwicklung der Niere." Jena, Gustav Fischer, 1927.
6. Mollenhauer, H. H. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* 39, 111, (1964).
7. Trump, B. F., Smuckler, E. A., and Benditt, E. P. A method for staining epoxy sections for light microscopy. *J. Ultrastruct. Res.* 5, 343, (1961).
8. Androlo, V. T., and Epstein, F. H. In "Progress in Pyelonephritis" (E. H. Kass, Ed.), pp. 232–238. F. A. Davis Co., Philadelphia, 1964.
9. Beeson, P. B., and Rowley, D. The anticomplementary effect of kidney tissue. Its association with ammonia production. *J. Exp. Med.* 110, 685, (1959).