Blockade of Rennin-Angiotensin system blunts the fibrotic response in experimental acute pyelonephritis

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ABSTRACT

Aim: To study the impact of Renin-Angiotensin system blockade in experimental acute pyelonephritis, induced by a novel surgical approach via dorsal lumbotomy incision.

Materials and Methods: 45 Adult female WISTAR rats aged 8-12 weeks, underwent direct inoculation of 0.1 ml of E.coli suspension into the parenchyma of the surgically exposed kidney. 3 groups of rats were studied: Group A – treated with antibiotics only; Group B- Captopril and antibiotics and Group C- Losartan and antibiotics. Changes of acute inflammation, parenchymal destruction and scarring were compared between the groups on histopathological sections. Kruskal-Wallis test was used for statistical analysis.

Results: Changes consistent with acute pyelonephritis were seen in all the kidneys. Mean% scar area in Group A, Group B and Group C was 37.08±1.79, 24.40±1.88 and 24.68±1.32% respectively at end of six weeks. Mean tubular density in Group A, B and C was 17.26±1.92, 47.18±3.00 and 47.00±5.08-tubules/µm² respectively. The differences between the control and the treated animals were significant, though the results did not differ between the losartan and captopril treated rats.

Conclusions: Dorsal lumbotomy approach to the kidney provides a good exposure of the kidney. Induction of acute pyelonephritis by direct inoculation of bacteria into renal cortex produced a consistent scar at 6 weeks. Blockade of renin angiotensin system by either captopril or losartan decreased the renal scar area by almost 1/3 at 6 weeks.

KEY WORDS: Experimental pyelonephritis; renal scar; renin-angiotensin system; angiotensin converting enzyme inhibitor; angiotensin receptor blocker; lumbotomy incision

Acute pyelonephritis may lead to cortical damage and lead to sequelae such as scarring and repeated attacks may lead to renal failure.

Early diagnosis and timely institution of appropriate antibiotics may minimize the scar formation. It has been shown that late institution (> 72 hours) of antibiotic therapy may not prevent the scar formation effectively as the inflammatory response has already set in.[1-4] It is in this scenario that the role of adjunctive drugs to reduce the severity of scarring has been a topic of intense research. Experimental studies have examined the effect of addition of allopurinol,[2] corticosteroids,[3,5] antioxidants,[6] vitamins,[7,8] anti-inflammatory drugs,[9] angiotensin receptor blocker[10] and pentoxiphylline[4] to the antibiotic regimen with some success.

Renin-angiotensin system (RAS) is important for homeostasis and preservation of renal function in normal state. Activation of RAS, which occurs with nephron loss in an effort to preserve the glomerular filtration rate, proves to be maladaptive in the long run as it also promotes glomerulosclerosis.[11] The present study explores the impact of blockade of RAS of experimental APN.

MATERIALS AND METHODS

Animals
Adult female WISTAR rats aged 8-12 weeks, weighing 150-200 g were chosen for the study. The rats were housed one in each cage in an air-conditioned room at 20ºC and fed standard rat chow and Bengal grams. Captopril and Losartan were used in the dose of 10mg/kg/day. The tab-
lets were crushed to yield fine powder and then mixed in drinking water of rats.

**Induction of Acute Pyelonephritis**

E.Coli ATCC 25922 strain was used in colony count of $10^7$-$10^8$ for inoculation. This strain is pathogenic to rat and reliably produces acute pyelonephritis in rats.[8] The strain is sensitive to cefotaxime, which was used for treatment of APN.

APN was induced in rats by direct inoculation of 0.1 ml of *E. coli* suspension into the surgically exposed kidney parenchyma. After ketamine anesthesia the rats were laid prone and a dorsal lumbotomy incision was used to enter the peritoneal cavity. The kidney was delivered out and 0.1 ml of *E. coli* suspension was injected with a 26-gauge needle in the midpolar region at a depth of 2 mm (Figure 1a and 1b). The kidney was repositioned back and the muscles were closed in single layer with 4-0 vicryl sutures. Intraperitoneal Cefotaxime was given for 5 days at the dose of 200 mg/kg once a day, starting 72 hours after surgery.

**Grouping of Rats**

Three groups of rats were organised

Group A – 15 rats, bacterial inoculum given on day 1.
Group B – 15 rats, Captopril day 0, bacterial inoculum on day 1.
Group C – 15 rats, Losartan on day 0, bacterial inoculum on day 1.

4 rats from each group were operated at 48 hours and day 7 of bacterial inoculation and 7 rats from each group were operated at 6 weeks post-induction of APN.

Via the previous dorsal incision the right kidney was removed (Figure 1c). The renal capsule was stripped and gross changes were recorded. The kidneys were kept in 10% formalin solution till they were examined histopathologically.

**Histopathological Examination**

A standard section of kidney was taken vertically through the midportion of the kidney with 5 µm thickness. Sections were examined by a senior pathologist (AKD) who was blinded to treatment groups.

Degree of inflammation, abscess formation, bacterial colonies and the destruction of the pelvicalyceal system were graded on a scale of 1 to 3. At 6 weeks the scar area was evaluated and expressed as percentage of the total coronal area of the whole kidney mount. Tubular density was measured in the scar area to assess the destruction of the renal parenchyma and expressed as number of tu-

![Figure 1: (a) Operative picture depicting exposure of right kidney via modified lumbotomy incision; (b) Inoculation of bacterial suspension into renal cortex with a 26G needle in midpolar region; (c) Nephrectomy at 6 weeks via same incision, scarring is visible grossly also](image1.png)

![Figure 2: (a) Photomicrograph of Group A rat kidney at 6 weeks post induction of APN showing a large area of PCS destruction and fibrosis, in the lower half of the field fibrotic strands can be seen; (b) Photomicrograph of Group B kidney showing a comparatively smaller area of fibrosis; (c) Photomicrograph of Group C kidney showing reduction in area of renal scar. H/E, x2.](image2.png)
bules per lac µm².

Kruskal-Wallis test was used for statistical analysis with the significance level (P value) set at 0.05.

Ethical clearance for the experiment was taken from the Institute Animal Ethics Committee (IAEC 192/02) and all the procedures were conducted with sterile precautions and dignity towards animal life.

RESULTS

6 rats were excluded due to premature demise and thus the study was completed with 39 rats. 3 deaths occurred in the immediate post-operative period and were related to anesthesia overdose, the rest of the deaths were unrelated.

The rate of acute pyelonephritis and subsequent scar formation was 100% in the present study, signifying that this experiment yielded a consistent and predictable model of experimental renal scarring.

Changes at 48 hours
There was a discrete whitish focus of pyelonephritis at 48 hours post inoculation corresponding to the site of injection. The involvement often took a confluent linear shape extending along the anterior and the posterior surface to the hilum.

Histopathological examination showed acute inflammatory infiltrate with abundance of polymorphonuclear cells along with microabscesses, bacterial colonies and tubular destruction. There was no difference attributable to treatment with captopril or losartan.

Changes at 7 days
By 7 days the focus of pyelonephritis was slightly elevated over the kidney capsule. The microabscesses were seen in 5 animals only, and bacterial colonies had disappeared in all the animals signifying rapid response to antibiotics. The inflammatory infiltrate at 7 days consisted predominantly of mononuclear cells and lymphocytes implying chronic inflammation.

Changes at 6 weeks
The kidneys at 6 weeks showed characteristic depressed linear scars extending to hilum corresponding to the areas of APN. There was minimal chronic inflammatory infiltrate and there were no bacteria or microabscesses seen. The scar area was expressed as percentage of the total coronal section area (Figure 3).

Mean scar area in Group A, Group B and Group C was 37.08±1.79, 24.40±1.88 and 24.68±1.32% respectively. On intergroup comparisons, the scarring was reduced by 34% in captopril treated rats and by 33% in losartan treated rats when compared to control group (P=0.007). The differences between Group B and C were not significant.

Mean tubular density in Group A, B and C was 17.26±1.92, 47.18±3.00 and 47.00±5.08-tubules/lac µm² respectively. The differences between the control and the treated animals were significant (P value-0.006) though the results did not differ between the losartan and captopril treated rats. Data of the individual kidneys are presented in Table 1.

DISCUSSION

Renal scarring is a complex multifaceted process characterized by the proliferation of extracellular-matrix producing cells and excessive accumulation of extracellular matrix. Rat model of renal scarring is commonly used and for the induction of APN in rats, both retrograde and direct route can be used. As rats have uniformly 100% congenital reflux, bacterial inoculum into the bladder refluxes into the kidney and leads to APN. But either urethral obstruction for 4-6 hours[9,10] or inoculation under...
controlled pressure and at a constant rate is a must to ensure that this happens.[12] These conditions are difficult to standardize and still the scarring may be patchy.

On the other hand direct inoculation into a surgically exposed kidney leads to a discrete scar at the injection site and produces a more controllable situation.[4,8,13] For direct inoculation, midline incision in our experience was associated with more morbidity and even mortality. Our group has been using lumbotomy incision (access to kidney via dorsal incision) for all upper urological tract surgery in children with good results.[14] All these factors prompted us to try dorsal approach to kidney in rats also.

Culture sensitivity based antibiotic (cefotaxime) was given to all the rats starting after 72 hours. Timing of starting the antibiotic is critically important in determining the scarring. It is accepted universally that antibiotic treatment after 72 hours does not prevent significant renal scarring.[1-4] This protocol closely imitates the clinical situation in pediatric UTI as most of the time diagnosis is made late.

The changes seen at 48 hours and 7 days were not significantly altered by administration of captopril or losartan. The damage occurring at this stage is due to acute inflammation and probably both these drugs do not suppress this response.

Histopathological examination at 6 weeks showed wedge shaped scars in all the kidneys signifying consistent results. The reduction in scarring was consistent in all the specimens examined. Lesser PCS destruction was also an important finding. These results signify that renin-angiotensin system is definitely involved in pathogenesis and propagation of scarring in kidney.

Kavakcu et al administered vitamin A to rats in acute pyelonephritis and reported that there was significant reduction in inflammation, tubular atrophy and fibrosis.[8] Huang et al also employed a semi-objective scoring system for scarring in APN and reported significant reduction in scarring with combined use of antibiotics and ibuprofen, though either of the agents alone was not efficacious in decreasing the scarring.[9] Khalil et al reported the downregulation of TGF-beta with use of losartan in experimentally induced pyelonephritis.[10]

In the present study, no significant differences were observed on histopathological level between the control and the treated animals at 48 hours or 7 days. This signifies that the events are occurring at the molecular levels. A further study is planned to elucidate the mechanisms involved therein.

CONCLUSIONS

Dorsal lumbotomy approach to the kidney provides a good exposure of the kidney. Direct inoculation of a known bacterial strain into the renal cortex led to a predictable and reproducible focus of acute pyelonephritis, which subsequently evolved into a mature scar over a period of 6 weeks. Pre-treatment with captopril or losartan decreased the renal scar area by almost 1/3 at 6 weeks signifying that the renin-angiotensin blockade helps in decreasing fibrosis. The tubular density in the scar area was more in treated animals signifying less destruction of renal tubules. There was no difference observed in these effects between captopril and losartan implying that blockade of generation of angiotensin or its receptor were equally efficacious in this setting.

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