L-651,392, a Potent Leukotriene Inhibitor, Controls Inflammatory Process in *Escherichia coli* Pyelonephritis

MANON TARDIF, DENIS BEAUCHAMP, YVES BERGERON, CÉLINE LESSARD, PIERRETTE GOURDE, AND MICHEL G. BERGERON*

Laboratoire et Service d’Infectiologie, Centre de Recherche du Centre Hospitalier de l’Université Laval, and Département de Microbiologie, Faculté de Médecine, Université Laval, Ste-Foy, Québec, Canada G1V 4G2

Received 27 September 1993/Returned for modification 12 December 1993/Accepted 10 May 1994

In this study, the relationship between leukotrienes, peritubular cell infiltration with polymorphonuclear cells (PMNs) and renal tubular damage was investigated in a rat model of acute ascending pyelonephritis. Infection was induced by the injection of 10⁸ CFU of *Escherichia coli* into the bladder and occlusion of the left ureter for 24 h. Treatment of infected animals was started 24 h after the induction of pyelonephritis with either hydrocortisone (25 mg/kg of body weight per day), the leukotriene inhibitor L-651,392 (10 mg/kg/day), or the vehicle of L-651,392 and was maintained for 5 days. At the end of treatment, the animals were killed, serum was collected, and both kidneys were removed for colony counts and histopathology. Renal function was evaluated by the measurement of blood urea nitrogen levels and creatinine clearance. The numbers of PMNs and mononuclear cells (MNs) in the cortex and medulla were recorded for all groups on plastic sections done from the left kidney. Infection alone (vehicle of L-651,392) resulted in intensive interstitial infiltration and a severe tubular destruction in the cortex. Treatment with hydrocortisone did not prevent PMN migration and tissue damage. By contrast, treatment with L-651,392 resulted in a significant reduction in PMNs (~0.001 in comparisons with all other groups) and greater preservation of the tubular structure despite identical bacterial counts than in the group receiving hydrocortisone. We conclude that L-651,392 prevents inflammatory cells from reaching the site of infection and protects the kidney from tubular damage associated with inflammation during pyelonephritis. Inhibitors of leukotrienes should be further investigated for their potential benefit as adjuvants to antibiotic therapy in the treatment of pyelonephritis.

Several studies support the hypothesis that acute inflammation plays a major role in the development of tissue damage during infection. For example (17, 37), the presence of bacteria in the renal parenchyma during pyelonephritis induces a marked local cellular and humoral response. Inflammatory cells such as polymorphonuclear cells (PMNs) migrate into the interstitium under chemotactic stimuli and then release free oxygen radicals (O₂⁻, OH⁻, and H₂O₂) and lysosomal enzymes into their environment. Although these products are essential for bacterial killing, they are in part responsible for deleterious effects to host cells, including tissue damage and scar formation, with the ensuing decreased renal function as well as permanent kidney damage.

While the C5a fraction of complement may initiate the migration of PMNs in the early phase of the infection (38), the potent chemotactic leukotriene B₄ (LTB₄) and also the cysteinyll leukotrienes LTC₄, -D₄, and -E₄ are suspected of playing an important role in the outcome of pyelonephritis. Infiltrating leukocytes and cells from the rat glomeruli can produce leukotrienes (2, 11). Their synthesis is stimulated by bacterial components such as endotoxin. Studies of septic shock and different diseases of the lung have revealed that LTC₄, -D₄, and -E₄ can increase vascular permeability, resulting in severe edema (36). In experimental hydronephrosis and postischemic renal injury, LTB₄ has also been shown to stimulate cell influx (24, 42).

In this study, we have compared the protection afforded by hydrocortisone, a conventional glucocorticoid anti-inflammatory drug, with that of L-651,392, a specific 5-lipoxygenase inhibitor which interferes with the synthesis of LTBA, LTC₄, D₄, and E₄ (21) on the peritubular cell infiltration by PMNs and the integrity of the renal tubular structure during acute experimental ascending pyelonephritis.

**MATERIALS AND METHODS**

**Pyelonephritis model.** As the ascending route is the usual way by which bacteria reach and infect the kidney in human beings, acute retrograde pyelonephritis was induced in female Sprague-Dawley rats (weighing between 175 and 200 g) as previously described, with minor modifications (20). Animals were anesthetized with an intraperitoneal injection of chloral hydrate (350 mg/kg). The left ureter was exposed through a midline incision of the abdominal wall. At the midureter level, a polypropylene suture was passed through the flank into the peritoneal cavity and around the ureter. The ligature was left loosely in place while bacteria were injected into the bladder. As *Escherichia coli* is the causative organism in most of the renal infections, an inoculum of 10⁹ *E. coli* Yale strain cells of an overnight culture in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was gently infused, in a 0.3-ml volume of saline. Fifteen minutes later, the two arms of the ureteral ligature were tied through the left flank and the abdominal wall was closed. Twenty-four hours later, the animals were anesthetized and the ligature was cut from the outside and carefully removed. This model produces partial obstruction of urine flow and severe unilateral pyelonephritis in the left kidney. After 3 days, pyelonephritic kidneys are enlarged and display an intense inflammatory response, with numerous cortical...
abscesses (20). The virulent *E. coli* strain used in this experiment has also previously been shown to induce pyelonephritis and abscesses in other models of renal infection in the absence of ureteral obstruction (5–8).

**Treatments.** Infected animals were divided into three groups; 10 animals were treated with hydrocortisone (Solu-cor-tone; Upjohn, Mississauga, Ont, Canada); 10 treated with L-651,392 (Merck-Frosst Canada, Quebec, Canada) twice a day subcutaneously for a total of 25 mg/kg of body weight per day; 10 other rats received the orally active 5-lipoxygenase inhibitor L-651,392 (4-bromo-2,7-dimethoxy-3H-phenothiazin-3-one; Merck-Frosst Canada, Inc., Pointe-Claire, Quebec, Canada) at a dose of 10 mg/kg once a day by gavage; and 10 animals received the vehicle of L-651,392, which was made of 0.4% methylcellulose and 0.5% Tween 80. Infection was induced on day 1 and was maintained throughout the experiment. Urine was collected on day 4 of the experiment.

**Renal function.** Six animals from each group were individually housed in metabolic cages on day 4 of the experiment. Urine was collected over a 24-h period under mineral oil. The volume was measured, and the urine was centrifuged at 2,500 rpm (×11,400 g) for 15 min and kept frozen at −20°C. Creatinine in urine and in plasma obtained at the time of sacrifice was measured with a Stasar III spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Creatinine clearance (CLcr) was calculated as UV/P, where U is the concentration in urine, P the concentration in plasma, and V the urine flow per hour. Blood urea nitrogen (BUN) was measured in serum with a Biochromatic Analyzer ABA-100 (Abbott Laboratories, North Chicago, Ill.).

**Sacrifice of animals.** Animals were sacrificed on day 5 after induction of pyelonephritis. After anesthesia, animals were bled by cardia puncture and plasma was obtained by centrifugation. The abdomen was opened and both kidneys were removed aseptically, scored for gross pathological lesions, weighed, and bisected. Gross lesions were qualitatively quantified as follows: 0, uninfected kidney; 1 or 2, a few discrete macroscopical pinpoint abscesses; 3 or 4, several macroscopical pinpoint abscesses showing coalescence and occupying about half of the kidney surface; and 5 or 6, confluent macroscopical lesions occupying more than half of the kidney surface, with a severely scarred, atrophic, fibrotic, and undermined kidney. One half of the kidney was homogenized in 1.5 ml of saline at 4°C for bacterial enumeration, and the other half was processed for histopathology.

**Bacterial enumeration.** Serial dilutions of the kidney homogenates were rapidly done and plated in triplicate on McConkey agar (IAF Production Inc., Laval, Quebec, Canada). The plates were incubated for 18 to 20 h aerobically at 37°C, the resulting number of CFU was counted, and then the CFU per gram of tissue was calculated. Hydrocortisone and L-651,392 did not interfere with the assay.

**Histology.** Cubes of 1 mm² were taken from the cortex and outer medulla of the left kidney and left overnight in 0.5% glutaraldehyde at 4°C. After washing with phosphate buffer (0.1 M, pH 7.4), cubes were postfixed in osmium tetroxide (2%)–sucrose (4%)–PO₄ buffer, dehydrated in ascending grades of ethanol, and embedded in Epon 812 resin. Thick sections (1 μm) were cut with an ultramicrotome (Reichert-Jung Ultracut E; Vienna, Austria), stained with toluidine blue, and evaluated for the numbers of infiltrated PMNs and mononuclear cells (MNs) and cellular alterations in both the cortex and medulla. PMN and MN counts were expressed as the number of PMNs or MNs per high-power microscope field (400× magnification) per slide. Three slices from the cortex and from the medulla were analyzed for each animal. Thin sections doubly stained with uranyl acetate and lead citrate were also examined on a Philips 300 electron microscope for evaluation of damage to the kidney ultrastructure.

**Statistics.** Results were compared by an analysis of variance for repeated measures. Comparison of group means was done by Duncan’s multiple range test with Kramer’s adjustment for unequal frequencies (25).

**RESULTS**

Table 1 shows the incidence of gross lesions and percentages of the surface occupied by abscesses on the left infected kidneys. Significantly fewer lesions were observed on the surfaces of the kidneys of animals treated with L-651,392 than on those of animals treated with the vehicle of L-651,392 or hydrocortisone (*P* < 0.05). Similarly, the percentage of the surface occupied by abscesses was significantly reduced for the L-651,392-treated group compared with those for the two other groups (*P* < 0.05). The weight ratio of the left infected kidney (where edema occurred) to the uninfected right kidney was 1.95 for infected animals receiving no anti-inflammatory drug (vehicle of L-651,392). This ratio was not reduced significantly after treatment with either hydrocortisone or L-651,392, although a slight decrease was observed in both groups. Enlargement of the kidney, as measured as the ratio of the CLcr rate to body weight and the L/R wt ratio, was also examined for each animal. The ratio of CLcr was calculated in milliliters per minute per kilogram of body weight.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Gross surface lesion score (SEM)</th>
<th>% Kidney surface occupied by abscesses (SEM)</th>
<th>L/R wt ratio (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>3.7 (0.6)</td>
<td>65 (12)</td>
<td>1.95 (0.20)</td>
</tr>
<tr>
<td>H</td>
<td>2.9 (0.8)</td>
<td>54 (15)</td>
<td>1.86 (0.23)</td>
</tr>
<tr>
<td>L</td>
<td>1.5 (0.6)a</td>
<td>21 (10)b</td>
<td>1.77 (0.15)</td>
</tr>
</tbody>
</table>

a Treatment was started on day 2 with the vehicle of L-651,392 (V), hydrocortisone (H), or the 5-lipoxygenase inhibitor L-651,392 (L). *p* significantly different from the two other groups (*P* < 0.05). The number of animals examined was 10 per group. Normal uninfected rats (data not shown on the table) had no lesions, no abscesses, and an L/R weight ratio of 0.99 ± 0.03.

b Gross lesions were qualitatively evaluated on a scale from 1 to 6.
TABLE 2. CFU per gram of tissue in the left infected kidney and MNs in the left cortex and medulla 5 days after induction of E. coli pyelonephritis

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Log CFU/g (SEM)</th>
<th>MN/renal tubule ratio (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortex</td>
<td>Medulla</td>
</tr>
<tr>
<td>V</td>
<td>9.5 (0.6)</td>
<td>2.63 (0.67)</td>
</tr>
<tr>
<td>H</td>
<td>7.0 (0.6)*</td>
<td>4.31 (1.91)</td>
</tr>
<tr>
<td>L</td>
<td>6.9 (0.6)*</td>
<td>1.55 (0.37)</td>
</tr>
</tbody>
</table>

* Treatments were started on day 2 with either the vehicle of L-651,392 (V), hydrocortisone (H), or the 5-lipoxygenase inhibitor L-651,392 (L). * significantly different from the V group (P < 0.05); ** significantly different from the H group (P < 0.05).

Values are expressed as the number of MNs per tubule after counting of cells and tubules in three slices from the cortex and the medulla for each animal. Monocytes were essentially absent in kidneys from normal uninfected rats.

itor, in both the cortex and the medulla (Fig. 1). By contrast, no reduction of MNs or PMNs was observed in the renal tissue of animals given hydrocortisone. None of the normal uninfected rats revealed any evidence of acute inflammation.

Histological examination of the infected kidneys revealed similar anomalies in infected rats treated with the vehicle of L-651,392 and in those treated with hydrocortisone: an intensive infiltration of PMNs and MNs in the interstitium and tubular lumen of the cortex and medulla associated with enlargement of the interstitium and the presence of cellular debris (Fig. 2A). Electron microscopy (Fig. 3A) revealed additional injuries, including PMN infiltration through the tubular epithelia and changes in renal tubular cells such as mitochondrial swelling and irregular morphological changes of the nuclei and of the tubular basement membrane.

By contrast, peritubular infiltration by inflammatory cells was significantly reduced by L-651,392 (Fig. 2B). No inflammatory cells were seen in the lumina of these tubules. There was less enlargement of the interstitium than for the other groups. Moreover, these changes were associated with better structural preservation of the tubules. This protection was further confirmed by electron microscopy (Fig. 3B), since proximal tubules were easily differentiated and showed well-preserved brush border membranes, mitochondria, and nuclei. Lysosomes looked normal in all groups.

**E. coli** cells were easily seen on ultrathin sections in both the renal cortex and the medulla. They were essentially localized into vacuoles of PMNs in the lumina of tubules.

FIG. 2. (A) Peritubular cell infiltration in the infected left medulla of animal treated with hydrocortisone. Similar infiltration by PMN cells (arrow) and enlargement of the interstitium (I) between capillaries (C) and tubules (T) were seen in infected animals receiving no anti-inflammatory drug (vehicle of L-651,392). (B) Infected medulla of the left kidney treated with L-651,392. The tubular structure was well preserved, and cell infiltration was greatly diminished compared with that for infected animals receiving no anti-inflammatory drug or hydrocortisone. Magnification (A and B), ×1,020.

DISCUSSION

In this study, the relationship between renal infection, the inflammatory response, destruction of the tubular structure, and protection afforded by a leukotriene inhibitor was studied in an acute model of pyelonephritis. The results provide evidence that L-651,392 can suppress peritubular cell infiltration and protect the kidney from damage.
Renal infection in this model induces injuries similar to those previously reported by other investigators (41), with bacterial proliferation and inflammatory response resulting in edema, tubular destruction, and gross morphological lesions in the infected kidney. As reported previously, both monocye and neutrophil infiltrations were observed (13, 32).

We have observed a direct correlation between leukocyte tubular infiltration and tissue destruction. In fact, both macrophages and neutrophils may have liberated a variety of cytokines (tumor necrosis factor and interleukins), metabolites of arachidonic acid, and noxious inflammatory mediators such as oxygen radicals and lysosomal enzymes during the process of phagocytosis, which may have accounted for the deleterious effects to kidney cells. While inhibition of acute suppuration by cyclophosphamide-induced neutropenia (9) or antioxidant therapy with radical scavengers such as superoxide dismutase (37) and even complement depletion with cobra venom factor (38) have all been reported to reduce kidney damage after infection, the present study reports a major protective role for a leukotriene inhibitor in pyelonephritis.

In our experiment, both hydrocortisone and L-651,392 slightly decreased the number of viable bacteria in renal parenchyma on day 5 after the induction of infection. While an increase in the proliferation of bacteria has already been observed after treatment with dexamethasone or cyclophosphamide as a result of immunosuppression (33), other investigators have reported a reduction in colony counts after indomethacin treatment (19). We believe, like these authors, that the reduction in bacterial counts observed in our study can be attributed to better clearance of bacteria in urine due to reduced suppuration and obstruction following administration of anti-inflammatory drugs.

Since our animals were sacrificed on day 5, it was not surprising to observe the absence of a significant reduction in the weight of the infected kidney after hydrocortisone treatment, because the anti-inflammatory activity of glucocorticoids during severe infection is very short-lived (31). As for L-651,392, our results suggest that it had a profound effect on cell migration within the tubules and renal parenchyma but did not, as determined by the very limited reduction in the left-to-right kidney weight ratio, totally inhibit the increase in vascular permeability and marked edema associated with gram-negative infections.

However, despite identical bacterial counts in the renal parenchyma, hydrocortisone- and L-651,392-treated animals responded quite differently to treatment. Hydrocortisone failed to prevent leukocyte infiltration and to reduce tissue damage. By contrast, a sharp reduction in inflammatory cells associated with a marked cytoprotective effect was seen with L-651,392 even 5 days after the induction of infection. This difference provides further evidence that kidney damage results to a large extent from the inflammatory reaction rather than from bacterial infection alone and that leukotrienes play a major role during pyelonephritis.

The failure of hydrocortisone to prevent leukocyte infiltration and to reduce the pathological process despite the known capacity of glucocorticosteroids to diminish production of cytokines and arachidonic acid and to stabilize membranes (30, 34) is consistent with the results reported by Meylan and Glauser (31). They reported a reduction in PMN migration very early (24 h) during the development of an acute exudative pyelonephritis treated with dexamethasone, but this effect vanished with the increasing inflammatory response, despite adequate dosage and schedule of administration.

Our study stresses the importance of selective leukotriene inhibition during pyelonephritis. L-651,392 is an orally active (16), in vivo-effective and -selective 5-lipoxgenase inhibitor (21). This compound was shown in rat, sheep, and monkey models of endotoxemia and of diverse pulmonary diseases (12, 28, 29) to interfere with LTβ, and LTC4, -D4, and -E4 synthesis. LTβ, which is produced by glomerular mesangial cells (11, 18), has been associated with PMN infiltration (15, 27, 43) and macrophage attachment (1) in glomerulonephritis. In our animal model of pyelonephritis, LTβ inhibition may have contributed to diminishing early leukocyte migration and to reducing tissue damage, thus also limiting further influx of PMN and release of additional leukotrienes and cytokines from inflammatory cells. The inhibition in leukocyte chemotaxis may have occurred directly or indirectly, since cytokines

![Image A](http://aac.asm.org/)

![Image B](http://aac.asm.org/)

**FIG. 3.** (A) Electron micrograph of the infected medulla in the left kidney of animal treated with hydrocortisone. E. coli (arrow) cells are seen in the cytoplasm of PMNs (P). The same observations were made with animals receiving the vehicle of L-651,392. (B) Electron micrograph of the left infected medulla treated with L-651,392. The tubular ultrastructure was well preserved. B, brush border; M, mitochondria; N, nucleus; I, interstitium. Magnification (A and B), ×11,628.
have been reported to mediate some of the leukotriene effects (40).

Inhibition of LTC₄, -D₄, and -E₄ may also have contributed to the preservation of renal hemodynamics and tubular integrity. LTC₄, -D₄, and -E₄ have been shown to decrease the glomerular filtration rate by inducing vasoconstriction (39) and to increase capillary permeability (22). These leukotrienes act as mediators of mesangial cell injury (10) and acute renal failure (35) during drug-induced nephropathies. Although renal function assessed by serum BUN and Cl-Cr remained essentially unaltered in our experiment, we do not exclude the possibility that the right uninephrectomized kidney compensated for any microvascular injury or any reduction in the glomerular filtration rate that could have occurred in the left untreated infected kidney. Moreover, in this model, as in acute pyelonephritis in humans, there is usually very limited damage to the glomeruli so that Cl-Cr is rarely affected. Since LTC₄, -D₄, and -E₄ have been considered mediators of renal functional impairment in experimental endotoxemia (4) and since endotoxin is liberated locally and systemically during the infectious process, the use of L-651,392 probably also protects the kidney from functional impairment in models in which a larger inoculum or a more virulent strain is used to induce significant alteration at this level.

Recent data from other investigators (1, 3, 15) strengthen the concept that leukotriene inhibitors are useful drugs during overwhelming inflammation in kidney disease. Their data support the notion that naturally occurring substances (the 15-lipoxygenase derivatives 15-S-HETE [15(S)-hydroxyeicosatetraenoic acid] and lipoxins) act as counterinflammatory signals to antagonize the proinflammatory actions of the leukotrienes (5-lipoxygenase derivatives), thus providing protection against glomerulonephritis.

In our experiment, L-651,392 proved useful in a pyelonephritis model, thus confirming its in vivo-effective properties after oral administration. Although some other leukotriene antagonists or inhibitors have already shown protection against death in animal models of endotoxemia (14, 23), these drugs deserve investigation in pyelonephritis models in which, in view of the present findings, they show remarkable protective effects and in which they could be used as adjuvant therapy to antibiotics and could hopefully protect against chronic pyelonephritis.

ACKNOWLEDGMENTS

This study was supported by grants from the Kidney Foundation of Canada and the Medical Research Council (MA-10157) of Canada. D.B. is the recipient of an F.R.S.Q./Eli Lilly scholarship.

We thank Merck Frosst Canada, Inc., for providing L-651,392 and Nathalie Ouellet, Pierre Provencher, Brigitte Lambert, and Odette Guibord for their valuable assistance.

REFERENCES


