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Original Article

Effects of Betamethasone and Gentamicin on Renal Scarring Induced by Mannose-Sensitive *E Coli* Pyelonephritis in Rat

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Abstract

Background: Pyelonephritis can lead to renal scar.

Objective: To evaluate the effects of betamethasone in preventing renal scarring in rat model of pyelonephritis.

Methods: Sixty three female Sprague-Dawley rats were divided into seven groups. Group A was the control. Mannose-sensitive *E coli* was directly inoculated into the left kidney exposed under general anesthesia in groups C–G. Group B received normal saline. Two days after bacterial inoculation, groups C, E and F received gentamicin for 10 days. Betamethasone was injected for three days to animals in groups of D, E (2 days after bacterial inoculation) and F (5 days after bacterial inoculation). Group G received no treatment. Eight weeks after bacterial inoculation, animals were sacrificed and the volume (amount) of renal scar was determined using the stereological techniques.

Results: Changes in the weight and volume of the kidneys were not statistically significant. No scar was detected in group A, but all other groups including group B with intrarenal injections showed scarring. The volume density and absolute volume of the scar in groups C–G were significantly more than group B ($p<0.001$), whereas they did not differ significantly from each other.

Conclusion: Betamethasone and/or gentamicin, when used two days after induction of pyelonephritis were not effective in preventing renal scar.

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Keywords: Pyelonephritis ● Renal Scar ● Betamethasone ● Gentamicin ● Rat

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Introduction

Pyelonephritis can lead to scar formation in the kidney that in turn may cause renal dysfunction and/or hypertension. Indeed, renal scar is one of the most important complications of acute pyelonephritis. Although numerous clinical and experimental studies have demonstrated that the rate of renal scarring following documented pyelonephritis can be diminished with appropriate antibiotic therapy, approximately 40% of the cases will still develop renal scar.[1] It is helpful to find an adjuvant therapy that would prevent renal scar after

an episode of acute pyelonephritis. One group of drugs with potent anti-inflammatory effects are glucocorticoids. These drugs down-regulate cell-mediated immunity by inhibiting cytokine release, migration of leukocytes, cell-to-cell signaling and the production of destructive enzymes.[2]

Oral glucocorticoids have previously been used to prevent scar formation in animal models of pyelonephritis.[2,3] To the best of our knowledge, the long-acting injectable form of these drugs has not been tested so far. This study therefore was undertaken to determine the effect of intramuscular injection of betamethasone on prevention of scarring in an animal model of pyelonephritis.

Materials and methods

Animals

This study included 63 female Sprague-Dawley rats, 6–8 weeks old, weighing between 200 and 250g. They were maintained at room temperature on pellet rat diet with tap water available *ad libitum*. The rats were randomized into seven groups (A to G) each including nine animals.

Bacteria

Mannose-sensitive *Escherichia coli* (sensitive to gentamicin) were isolated from the urine sample of a patient with urinary tract infection and Mannose-sensitive *E coli* was confirmed by hemagglutination test.

Bacterial inoculation

The rats were anesthetized with intraperitoneal injection of ketamine HCL (100mg/kg) and xylasine (10mg/kg). Subcostal incision under costal margin was made on the left side, in order to expose the left kidney. In groups C to G, using a 24 G needle, 10^9 colony forming unit of *E. coli* in 0.1 ml saline was directly inoculated into the parenchymal tissue of the left kidney.[4] Rats in group B received normal saline whereas those in group A received no injection.

Treatment protocols

Group A received no treatment. Group B only received a volume of normal saline equal to treated groups. Two days after bacterial inoculation groups C, E and F received gentamicin (3 mg/kg/day IM) for 10 days. Betamethasone (0.3 mg/kg/day IM) was injected for three days to animals in group D and E (2 days after bacterial inoculation) and F (5 days after bacterial inoculation). Group G received no treatment.

Stereological estimation of renal scarring

After 8 weeks, all rats were anesthetized with ether and perfused trans-cardially with a solution of neutral buffered formaldehyde. The left kidney was then removed and the volume of each kidney was determined by immersion method. The kidneys were maintained in neutral formaldehyde for one week. After tissue processing, using a microtome, each kidney was thoroughly cut into coronal sections. The tissue sections (five micrometers thickness) were chosen randomly with an interval of 500 micrometers and stained with Heidenhain's azan. The volume density of the scar tissue (the fraction of the renal tissue that is occupied by the scar) was assessed using a point-counting technique at a final magnification of ×50 under the projection microscope (Visopan, Austria). Briefly, a collection of points superimposed on the projected images of the renal tissues. The percent of scar tissues (volume density) were obtained by dividing the number of points over the scar tissues by the total number of points on the reference space. Absolute volume (amount) of scar tissues was also estimated by multiplying the volume density by the kidney volume.

Statistical analysis

Data are presented as Mean±SD. Student *t* test was used for all comparisons and $P<0.05$ was considered as statistically significant.

Results

The kidney weight, volume, volume density and absolute volume of the scar in different groups are shown in Table 1. There were no statistically significant differences among groups regarding kidney weight and volume. No scar tissues were observed in group A. All other groups that had intra-renal injections with either bacteria or normal saline showed scar tissue. The volume density and absolute volume of scar tissues of groups C–G were significantly higher than group B ($p<0.001$), whereas they were not significantly different from each other.

Discussion

This study has used stereological methods to demonstrate the effects of betamethasone on renal scaring induced by Mannose-sensitive *Escherichia coli* in rat. Renal scar is an important complication of acute pyelonephritis. Although numerous clinical and experimental studies have demonstrated that the rate of renal scarring, following pyelonephritis, can be diminished using proper antibiotics, with ap-

Table 1: Data of the kidney weight (KW), volume (KV), volume density (VD) and absolute volume (AV) of the scar tissue

Groups	A (n=9)	B (n=9)	C (n=9)	D (n=9)	E (n=9)	F (n=9)	G (n=9)
KW (g)	0.82 ± 0.10	0.83 ± 0.09	0.80 ± 0.08	0.83 ± 0.08	0.85 ± 0.14	0.83 ± 0.12	0.77 ± 0.15
KV (ml)	0.78 ± 0.11	0.74 ± 0.06	0.76 ± 0.10	0.77 ± 0.09	0.72 ± 0.14	0.77 ± 0.08	0.64 ± 0.12
VD (%)	0	0.28 ± 0.22*	4.33 ± 3.67	7.39 ± 6.40	4.92 ± 4.57	5.25 ± 5.62	5.60 ± 4.65
AV (ml)	0	0.002 ± 0.001	0.03 ± 0.02	0.03 ± 0.03	0.04 ± 0.04	0.04 ± 0.04	0.03 ± 0.02

Values are Mean±SD

* P< 0.001, group B *vs.* groups C to G

proximately 40% of cases still having renal scars.[1] Some authors have noted that renal scarring is attributed to the inflammation or suppuration that follows bacterial infection rather than to the bacterial growth in the kidney. In this respect, different drugs have been tried to suppress inflammation.[2-10]

The mechanism of scar formation has been explained by several researchers.[4,11,12] It is shown that the host-derived cytotoxins are central to the genesis of tissue suppuration and several authors have tried to understand and modify the inflammatory response.[4,12] The role of reactive oxidants, nitric oxide and tissue destructive proteinases in the acute inflammatory response has recently been documented.[11,12] It has been demonstrated that even the presence of small amounts of oxidants activate proteinases enzymes, and will significantly destroy renal tissues.[11,12] Circulating proteinase inhibitors are normally present in high quantities and safeguard against the destructive potentials of these enzymes. Thus, in the absence of severe inflammation, any enzyme released from neutrophils are immediately inactivated. However, with neutrophilic stimulation, the aforementioned oxidants are released into the immediate vicinity and create a microenvironment capable of inactivating enzyme inhibitors, activate latent neutrophil-derived enzymes, and rapidly degrade microbes, host cells and connective substratum.[13] The inflammatory process continues and extends through the renal interstitium, leading to a damage that ultimately results in irreversible renal scarring.[2]

Glucocorticoids, through a negative effect on cytokine expression, can inhibit expression of cell surface adhesion molecules and release chemo-attractants, thus muting the neutrophilic response to inflammation.[13] Glucocorticoids can also induce transcription of lipocortin-1, a protein that inhibits the production of leukotrienes, which are metabolites of phospholipids implicated in ischemia-reperfusion injury.[13] Thus, by modulating a critical step in the initiation of inflammation, glucocorticoids might be beneficial in the prevention of pyelonephritis-induced renal scarring.

In previous animal studies, oral glucocorticoids were used with some beneficial effects in preventing renal scars.[2,3] In the present study, no significant inhibitory effects of renal scarring were found when betamethasone and/or gentamicin was administered two days after bacterial inoculation. The absolute amount of scar tissues were 0.03-0.04 ml in betamethasone and/or gentamicin treated rats and none of these drugs could prevent scar formation. A possible reason for the difference between the results of our study and those of others might be the route of induction of pyelonephritis. Glauser et al and Bille et al induced ascending pattern of pyelonephritis,[1,6] whereas in this study bacteria was injected directly into the kidney. The other reason might be the initiating time of the treatment. In the above mentioned investigations the treatment started immediately after pyelonephritis induction, whereas in the present study this was started two days after induction of pyelonephritis, as patients usually do not visit their physicians immediately after infection.

It is concluded that treatment of pyelonephritis with betamethasone and/or gentamicin, two days after the bacterial inoculation will not prevent renal scarring in rat.

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