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What Is the Risk of Pyelonephritis Due to Desmopressin in Rats with Cystitis?

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SUMMARY

The risk of pyelonephritis in children with asymptomatic cystitis or bacteriuria, using desmopressin for primary nonpoliuric nocturnal enuresis, is not known. The aim of this study was to research whether there is a risk of pyelonephritis in rats with cystitis using desmopressin. Wistar rats (n = 28) were divided into four groups of cystitis (groups I–IV). DDAVP (2 μg daily) and saline (0.5 ml daily) were injected intramuscularly for 7 days in groups II and IV and groups I and III respectively. The urinalysis, urine culture, and 24-h urinary volume (UV_{24}) were assessed for all rats on days 1, 3, 5, and 7. In groups III and IV these studies were also performed on days 14, 21, and 28. Serum creatinine was determined on day 7 in all rats and on day 28 in groups III and IV. Groups I and II and III and IV were killed at the end of days 7 and 28 respectively. Kidneys and urinary bladders were graded subjectively for inflammation and fibrosis. Inflammation and fibrosis scores in kidney and bladder tissues were not different between DDAVP or saline injected rats in cystitis groups at first and fourth weeks. No fibrosis was found in any of the urinary bladder through histological examination. Ascendant pyelonephritis was detected in each of the four rats in DDAVP-administered and saline-administered cystitis groups. The histopathologic scores of the renal tissue with pyelonephritis showed no correlation with the daily urine volume, the positive test results for urine leukocyte esterase with dipstick test, the urine culture results for E. coli *based on colony forming unit per milliliter, or serum creatinine levels in cystitis groups. It was found that the administration of DDAVP to cystitis groups did not increase the risk of ascendant pyelonephritis.* © 2006 Prous Science. All rights reserved.

Key words: Cystitis - Desmopressin acetate - Pyelonephritis - Rat

INTRODUCTION

It is known that pyelonephritis during the childhood usually occurred by ascendant uropathogenic microorganisms. Cystitis as a lower urinary tract infection (UTI) is also seen frequently in children. Clinical picture of cystitis may be asymptomatic. Lack of enough drinking and lower urinary volume is one of the causes for UTIs (1-3).

Desmopressin is one of the pharmacological agents for primary nocturnal enuresis (4-8). Although it has been suggested that desmopressin has a role in treatment of poliuria due to decreased ADH during the night, in recent years the role of desmopressin on central nervous system by decreasing the threshold of awakening for emptying the bladder has been shown. (9). On the other hand, desmopressin used for treatment of primary nocturnal enuresis may be the reason for upper UTI due to decreased urinary volume in nonpoliuric children with enuretic and asymptomatic cystitis. The risk of pyelonephritis in children with asymptomatic cystitis or bacteriuria, using desmopressin for primary nonpoliuric nocturnal enuresis, is not known.

The aim of this study is to research whether there is a risk of pyelonephritis in rats with cystitis using desmopressin.

MATERIALS AND METHODS

The study comprised 28 male Wistar rats (6–8 weeks old; body weight, 200–290 g) maintained at room temperature with drinking water and fed with standard rat pellets *ad libitum* throughout the study. Sepsis, local infection, increased catabolism, and trauma are defined as exclusion criteria for rats. At the beginning of the study, the urinalysis and bacterial urine cultures were performed with midstream void urine samples; urinary volume was assessed with the collected 24-h urine samples for all rats. Twenty-four-hour urine samples were collected in metabolic cages. Rats were held down and the midstream void urine was taken in sterile glasses. The leukocyte esterase of midstream void urine sample was also assessed with dipstick test; bacterial urine culture was made using Eosine Methylene Blue (EMB) and sheep-blood broth. Growth of *E. coli* was assessed according to colony counts per milliliter urine (cfu/ml);

a gram-positive bacterial growth was thought of as contamination.

If urine cultures were negative for *E. coli* at the beginning of the study, the experimental cystitis models were performed using a solution containing *E. coli* ATCC 25922 strain (10^{10} bacteria/ml) that was prepared and administered to the rats on the day it was prepared (10). The rats were anesthetized with inhalable halotan (induction with 2–3% halotan; maintenance with 0.5–1.5% halotan) while they received 100% oxygen (11). Under anesthesia, 28 rats underwent urethral catheterization and intravesical instillation of 0.3 ml of the same bacterial solution (12). Under anesthesia, periurethral region was sterilized with 70% ethanol. The urethra was then catheterized with a sterile 22-gauge polyethylene spinal catheter (Portex™), the bladder was emptied, and then the bacterial solution was instilled as described previously, but the urethra was not occluded with colloidum in our study (12). The urine culture was obtained 1 day after application of the bacterial inoculation. When the urine culture was negative for *E. coli*, the same bacterial inoculation procedure was repeated. After the urine culture became positive for *E. coli*, four groups (I–IV), each consisting of seven rats, were formed. All four groups had cystitis.

Two micrograms of DDAVP was selected as an antidiuretic dose defined previously (13-15). In groups II and IV, a dose of 2 μg DDAVP (Minirin®, 4 μg/ampoule; ER-KIM Ilac Sanayi ve Ticaret, A.S., Izmir, Turkey) per day, for 7 days, was injected intramuscularly, starting 1 day after bacterial inoculation; in groups I and III equal volume of saline solution (0.9% NaCl, a dose of 0.5 ml/day for 7 days) was injected intramuscularly, starting 1 day after bacterial inoculation.

The urinalysis, bacterial urine cultures, and urine volume were assessed for all rats at first, third, fifth, and seventh days of DDAVP or saline injection; additionally, in groups III and IV, the studies were also performed at 14th, 21st, and 28th days (Table 1).

Eight days after bacterial inoculation to the rats in groups I and II and 29 days after bacterial inoculation to the rats in groups III and IV, the rats were anesthetized and killed. After obtaining 5 ml of blood, under anesthesia, from the abdominal aorta, both kidneys and urinary bladder were extirpated and stored in 10% formalin solution for histopathological evaluation (10, 12). The obtained blood samples were used for determination of serum creatinine level. Additionally, under anesthesia, blood samples (1 ml each) of rats in groups III and IV were obtained from vena caudalis at seventh day of DDAVP or saline injection. Serum creatinine levels were determined using the Jaffe method by autoanalyzer of Hitachi P-800 (Roche Diagnostic, Izmir, Turkey).

The formalin-fixed kidneys and urinary bladder of each rat were evaluated by a pathologist who was unaware of the group of rats. Sections (4 mm thick) from both kidneys and urinary bladder were placed on slides and stained with hematoxylin and eosin (H&E) and modified Masson's trichrome (mMT); they were then examined histopathologically by light microscopy, for inflammation and fibrosis, and were graded subjectively (10, 12).

The urinary bladder inflammation was scored subjectively on the H&E-stained sections as 0 (not present), 1 (mild), 2 (moderate), and 3 (severe) (16). Urinary bladder fibrosis was also scored subjectively on mMT-stained sections as 0 (not present) and 1 (present). The renal inflammation and fibrosis were evaluated on the H&E- and mMT-stained sections respectively (10, 12).

The Mann–Whitney *U* test was used to compare the mean UV_{24}, *E. coli* colony counts in bacterial urine cultures, serum creatinine levels at the end of 7th and 28th days, and histopathologic scores; Fischer's χ^2 test was used to compare urine leukocyte esterase results. The correlation analysis was used to compare the histopathologic scores with urine volume, urinary leukocyte esterase results, *E. coli* colony counts in urine cultures, and serum creatinine levels.

RESULTS

One rat in group I was excluded from the study because the growth of *E. coli* had been detected in urine cultures at the beginning. The mean weight of rats was 226.88 ± 29.61 g. The variables of the rat groups were evaluated in the first week according to the same procedures employed in the combined groups. UV_{24} during the first week of the research program implemented was lower in DDAVP-injected cystitis groups than in the saline-injected cystitis groups (Table 2). This difference disappeared after the seventh day.

The urine leukocyte esterase, bacteriuria, and the daily urine culture results for *E. coli* (cfu/ml) did not show any difference between DDAVP- and saline-injected rats in cystitis groups The mean (minimum –

TABLE 1. Summary of the groups and the employed procedure

Group[a]	Intramuscular injection	Days on which experimental analyses were done	Day of killing
I	Saline (0. ml/day) for 7 days	1, 3, 5, 7	7
II	DDAVP (2 μg/day) for 7 days	1, 3, 5, 7	7
III	Saline (0. ml/day) for 7 days	1, 3, 5, 7, 14, 21, 28	28
IV	DDAVP (2 μg/day) for 7 days	1, 3, 5, 7, 14, 21, 28	28

[a]The rats in all groups had cystitis.

TABLE 2. Results of the comparison of mean UV_{24} by Mann-Whitney *U* test

Day	UV_{24} (ml)		Mann-Whitney *U* test (M-W *U*)	*p*
	Groups II and IV (DDAVP injected)	Groups I and III (saline injected)		
1	4.90 ± 1.7	7.28 ± 2.99	46,500	0.029
3	4.74 ± 1.57	10.52 ± 4.60	22,000	0.0
5	4.83 ± 1.08	9.61 ± 3.05	13,500	0.0
7	5.61 ± 1.83	8.54 ± 2.75	33,500	0.007

Values given are mean ± SD.

maximum) serum creatinine levels at 7th and 28th days were 0.62 ± 0.007 (0.44–0.75 mg/dl) and 0.66 ± 0.007 (0.50–0.78 mg/dl) respectively. The serum creatinine levels were found in normal ranges for all rats at 7th and 28th days of the study (17, 13).

Inflammation and fibrosis scores in kidney and bladder tissues were not different between DDAVP- or saline-injected rats in cystitis groups at first and fourth weeks. No fibrosis was found in any of the urinary bladder through the histological examination. (Fig. 1 is an example of the inflammation of the urinary bladder sections). Ascendant pyelonephritis was detected in each of the four rats in DDAVP- and saline-administered cystitis groups.

The histopathologic scores of the renal tissue with pyelonephritis showed no correlation with the daily urine volume, the positive test results for urine leukocyte esterase with dipstick test, the urine culture results for *E. coli* based on colony forming units per milliliter, or serum creatinine levels in cystitis groups.

DISCUSSION

Although enuresis and recurrent UTI are common problems in childhood, and DDAVP is used for some enuretic patients, no report was found about the role of DDAVP on UTI in the current medical reports. In primary nocturnal enuresis due to desmopressin insufficiency during the night, desmopressin treatment compensates its deficiency, and so children's increased urinary volume becomes normal.

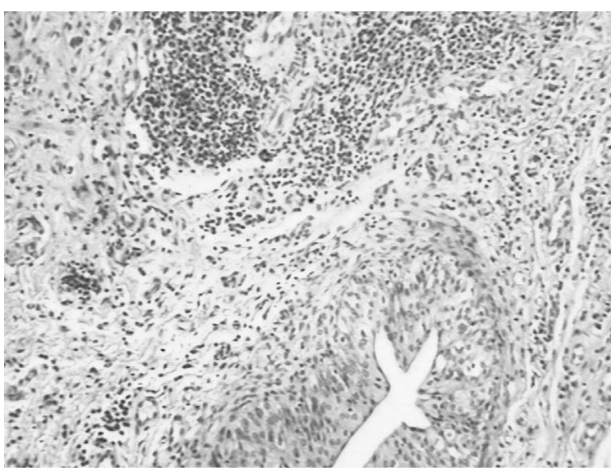


FIG. 1. Urinary bladder section. Severe inflammation is seen in this field. Other examination fields were also found to be similar to this field. The inflammation of this urinary bladder is subjectively scored as 3 (H&E-stained, ×10).

In primary nocturnal enuresis, desmopressin treatment decreases high volume of urine due to ADH insufficiency during the night. Therefore, the treatment should not be a risk for UTI. As per reports in the recent years, the effect of desmopressin has been showed as direct stimulation on central nervous system in children with primary nocturnal enuresis who had no poliuria due to ADH insufficiency during the night. In these children, urinary volume during the night naturally decreases. It is well known that low urinary volume is a risk for children with cystitis with regard to pyelonephritis due to arrival of uropathogenic agents from bladder to kidney easily (1-3). As we mentioned in the beginning of this report, the aim of this study was to research whether there is a risk of pyelonephritis in rats with cystitis using desmopressin.

A daily 2 mg fixed dose of DDAVP administered to Wistar rats that had cystitis resulted in antidiuresis. The effect of the antidiuretic dose of DDAVP on rats has been reported in previous studies (14, 15, 18).

The urine osmolality and density were not assessed in this study. Urine volume was used as a parameter to show the known effect of DDAVP on diuresis of the rats. It has been postulated that the decreased urine volume due to desmopressin would be a risk factor for ascendant urinary infection. Urine osmolality and density tests were not used for the follow up of the effect of desmopressin.

In this study it was, primarily, shown that a daily administration of 2 mg of DDAVP to rats that had cystitis had no effect on daily urine culture results for bacterial growth based on colony forming unit per milliliter; this procedure did not increase serum creatinine levels above normal ranges and had no effect on renal and urinary bladder inflammation and fibrosis process.

In conclusion, it was found that the administration of DDAVP to cystitis groups did not increase the risk of ascendant pyelonephritis. These findings revealed that the use of DDAVP in enuresis not due to higher urine volume did not appear to predispose the subject to UTI.

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